

Pharmacokinetics and Pharmacodynamics of Sustained,
Low-Dose Intravenous Infusions of Pyridostigmine

TASK ORDER #7

DRAFT FINAL REPORT

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Preface

This report was prepared at The Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, Maryland 21205, supported by the U.S. Army Medical Research and Development Command, Contract No. DAMD17-85-C-5133, Task Order #7, "Pharmacokinetics and Pharmacodynamics of Sustained, Low-Dose Intravenous Infusions of Pyridostigmine." This project was conducted in collaboration with the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. Col. Brian Schuster, M.D. of the Division of Experimental Therapeutics was the project monitor.

This work was conducted in The Johns Hopkins Hospital between 27 July and 16 October 1987.

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SUMMARY

Pyridostigmine bromide may be a useful adjunct to atropine sulfate to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. The inter-individual variability to a known, constant exposure to pyridostigmine is poorly characterized.

This study was designed (1) to assess the relationship between plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition, (2) to determine whether erythrocyte acetylcholinesterase and the contractile response of the iris to light are inhibited in a parallel fashion by pyridostigmine, and (3) to assess the inter-individual variations in the concentration-effect relationships described in 1 and 2.

The clinical portion of the study was conducted between 27 July and 16 October 1987, and showed that the constant intravenous infusion of low doses of pyridostigmine was safe and well tolerated. Intravenous pyridostigmine gave ~~constant peak~~^{steady} plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition. Mean peak erythrocyte acetylcholinesterase inhibitions were 29% and 36% for infusions of 12.5 mcg/minute and 18.75 mcg/minute, respectively. The mean rate constant of elimination from the central compartment was 1.365 hr^{-1} , corresponding to an elimination half-life of 30 minutes. The mean plasma clearance was 44.62 L/hr, or 744 ml/minute. The mean concentration at which 50% of the erythrocyte acetylcholinesterase activity was inhibited (IC_{50}) was 31.8 ng/ml. The influence of these infusions on the contractile response of the iris was difficult to measure and

was not consistent within subjects and no correlation with plasma pyridostigmine levels or acetylcholinesterase inhibition could be established.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects, the investigators have adhered to the policies of applicable Federal Law 45 CFR 46.

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1. INTRODUCTION

Studies in animals have indicated that carbamate acetylcholinesterase inhibitors are effective as adjuncts to atropine in protecting against organophosphate poisoning. Pretreatment of animals with carbamates and atropine prior to nerve agent exposure has improved survival and effectively increased the LD₅₀ of nerve gas agents (1). Experimental carbamate pretreatment is only effective when used in conjunction with atropine and is not adversely affected by oximes (1-4). Of the carbamates studied, pyridostigmine bromide has been found to be a useful agent with a duration of protective action of about four hours in guinea pigs following the intramuscular route of administration (2) and as long as 24 hours following oral administration of 1/9 the LD₅₀ of pyridostigmine in rabbits which were also supported by antidotal therapy (5).

The mechanism of action of pyridostigmine is thought to be carbamylation of a fraction of the tissue acetylcholinesterase, thereby protecting the enzyme from irreversible inhibition by the organophosphate (1-3, 5, 6). The relationship between carbamate drugs and the in vivo effects is a complex one which is not yet completely understood. Enzyme is inhibited in a pseudo-reversible manner; that is, the parent drug (pyridostigmine) first binds reversibly to acetylcholinesterase but then carbamylates the enzyme producing an inactive enzyme. The inactive carbamylated enzyme is reactivated when hydrolysis occurs, releasing active enzyme and dimethylcarbamate (7) (Figure 1). The inhibition of acetylcholinesterase following the administration of pyridostigmine can thus be expected to be dependent upon two factors: first,

the time course of drug in the body, i.e., the pharmacokinetics of the drug (8); and second, the sensitivity of the acetylcholinesterase enzyme to the drug, reflected in the relative rates of inactivation of enzyme and hydrolysis of the inactive enzyme in vivo.

We have previously studied 24 volunteers who received both oral and intravenous pyridostigmine (9). In that study it appeared that the acetylcholinesterase inhibition produced by pyridostigmine was delayed and prolonged relative to the plasma concentrations. Mathematical analysis of the data from that study suggested that a) the prolongation in inhibition is due to the relatively slow hydrolysis of carbamylated enzyme, b) both the pharmacokinetics of pyridostigmine and the sensitivity of acetylcholinesterase to pyridostigmine are highly variable between people, and c) the time course of drug administration is important since with slow hydrolysis significant enzyme inhibition will be achieved at low concentrations of drug.

The delay in enzyme inhibition which was observed suggests that effects due to enzyme inhibition outside the circulation may be delayed to a greater extent than erythrocyte acetylcholinesterase inhibition since more time may be needed for the drug to reach extravascular sites. Similarly, the acetylcholinesterase at the neuromuscular junction may not be identical to that in the erythrocyte. If so, similar degrees of inhibition at both sites may not be achieved by a given pyridostigmine concentration, i.e., the concentration-effect relation with pyridostigmine need not be the same at different sites. Since the clinical utility of the carbamates is probably related to protection of acetylcholinesterase other than the one in the red cell, it seems reasonable to assess the effect of pyridostigmine elsewhere.

One site where drug effect might be measured relatively simply is the iris of the eye which constricts in response to cholinergic stimuli.

Acetylcholinesterase inhibition thus should increase acetylcholine at the effector site producing decreased pupil size.

Prior studies demonstrated that pyridostigmine is removed rapidly from the body, making assessment of a concentration-effect relation difficult since drug levels decline rapidly and concentrations are low (8, 9). Furthermore, since hydrolysis of inactive enzyme is slow, the amount of enzyme inhibition at any time reflects the amount of drug that was present for the past one or two hours. Thus, slow infusion of pyridostigmine should be a better way to assess the relation between drug and effect. This method of delivery is safe as drug effects can be measured during the infusion and the infusion stopped if necessary. Using a slow intravenous infusion also allows drug effect to be measured when plasma concentrations are not changing rapidly. In this manner, the relation between drug and effect can be characterized with a minimum of experimental error.

The doses used in this study were calculated based on the following principles:

- a) Infusion should be of sufficient duration to produce steady state pyridostigmine concentrations and effects;
- b) Concentrations of pyridostigmine should be high enough to be accurately measured (> 10 ng/ml); and
- c) Inhibition of acetylcholinesterase at steady state should be 20% or higher.

The dose of pyridostigmine bromide and degree of acetylcholinesterase inhibition which provide adequate protection against organophosphate poisoning while at the same time minimizing unacceptable toxicity in man are not known. Studies in rats have indicated that inhibition of twenty-five percent of blood acetylcholinesterase does not affect muscle twitch tension, even after several days of therapy. On the other hand, abnormalities in twitch tension occurred in animals treated with enough pyridostigmine to produce sixty-eight percent inhibition of the enzyme (10). In addition, ultrastructural changes at the neuromuscular junction have been seen in rat diaphragm neuromuscular junctions at doses of pyridostigmine low enough to cause only about 10% reduction in blood acetylcholinesterase levels, and more severe damage was seen with doses of pyridostigmine causing 70% reduction in acetylcholinesterase activity. Changes from single doses of large amounts of pyridostigmine were present within 24 hours and appeared to be more extensive at 7 days after dosing. In animals given lower doses for 14 days, the changes appeared to be less severe and were largely reversible based on observations on animals sacrificed 7, 14, or 23 days after drug administration ended (11).

This study was designed (1) to assess the relationship between plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition, (2) to determine whether erythrocyte acetylcholinesterase and the contractile response of the iris to light are inhibited in a parallel fashion by pyridostigmine, and (3) to assess the inter-individual variations in the concentration-effect relationships described in 1 and 2.

2. MATERIALS AND METHODS

2.1 Pyridostigmine

Only one pyridostigmine bromide preparation was used in this study. This preparation was intravenous pyridostigmine bromide, produced by Hoffmann-LaRoche, Inc., Lot #0004, diluted in normal saline. Aliquots of the infusate were assayed for pyridostigmine base concentration. The pyridostigmine arrived at our facility on 26 July 1987. This test medication was provided by the U.S. Army. The supplies were kept in secure areas under lock and key in the Clinical Pharmacology Division complex of The Johns Hopkins Hospital or in the Pharmacy Department of The Johns Hopkins Hospital.

The intravenous pyridostigmine bromide (6000 or 9000 mcg) was diluted with normal saline to a total volume of 24 ml and infused at a constant rate over 8 hours by an infusion pump. Aliquots of the infusate were collected and frozen for later assay. The amount of pyridostigmine bromide administered to each subject is listed in Table 1. The amount is calculated from the results of the assays of each of the aliquots of infusate in the laboratory of Dr. Emil Lin at the School of Pharmacy, University of California at San Francisco, Contract No. DAMD17-86-C-6150 (12) and the rate of infusion of the infusate.

2.2 Subjects

Healthy men who were able to give written informed consent were eligible to volunteer for the study. The study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions and the Human Subjects Research Review Board of the U.S. Army.

2.2.1 Inclusion Criteria

To participate in the study the volunteer had to be male, between 18 and 35 years of age, and was to be within 10% of his ideal body weight as determined by Metropolitan Life Insurance Company tables (13). Equal numbers of whites and non-whites were to be recruited. Each subject was demonstrated to be in good general health based on a detailed health history and physical examination performed by a physician. No ophthalmological condition could be present that might interfere with the measurement or interpretation of pupillometry, and subjects had to be non-smokers. Serum chemistries, hematology tests, and urine analysis had to be within normal ranges, as defined by The Johns Hopkins Hospital Department of Laboratory Medicine. The protocol provided that the creatine kinase (CK) could be above the "normal range" and not exclude the subject because of the frequent finding of elevated CK in healthy subjects who are especially active physically (14-19). Nevertheless, at the request of the Army monitor for this study, the normal range according to the Hopkins laboratory was used as the acceptable range for the subjects in this study. An electrocardiogram within 12 months of entry had to be normal. The remainder of the screening evaluation was to be completed within 14 days of entry into the study.

2.2.2 Exclusion Criteria

Women were excluded from this study. Men were excluded if they did not meet the criteria listed above (2.2.1) or if they had a known or suspected allergy to pyridostigmine bromide or related drugs. Those with a history of significant heart disease, asthma, or other respiratory disorders were excluded. Once accepted as candidates for the study, subjects were not

permitted to take any medication for one week prior to admission to the study or during the study. A positive drug screen for opioids or cocaine on the day of admission excluded the volunteer.

2.2.3 Recruitment

Advertisements were placed in the help wanted classified sections of metropolitan Baltimore newspapers. A special telephone line was dedicated to volunteer recruitment. Interested candidates were screened on the telephone by a recruiter/screener who described the details of the study, took a brief history, and scheduled the appropriate screening examinations.

2.2.4 Informed Consent

Written informed consent was obtained from each participant. The consent document described in detail the purpose of the study, the research protocol, and the potential risks (Appendix A).

2.2.5 Compensation

A payment schedule was designed to compensate volunteers for their participation based on the number of days they were confined to the research unit, the number of doses of test medication given, and the number of blood samples taken. Each of the volunteers participating in Task Order #7 was to be remunerated \$360 for successful completion of the project. In an effort to complete the clinical portion of this project as efficiently as possible, we established (with the approval of the COTR) a program to provide a "back-up" volunteer to be admitted in the event a scheduled volunteer failed to arrive for admission to the study or if the scheduled volunteer failed screening blood tests or drug screens on the admission day. Each of the back-

ups not utilized but staying at the research unit all day until laboratory tests returned was compensated \$50.00. Volunteers asked to return for follow-up blood tests after the main portion of the project was completed were paid \$10.00 per visit. We also continued a "finder's fee" program, wherein individuals who successfully recruited other individuals into participation in the study were paid \$10.00 for each acceptable recruit.

2.2.6 Liability

Liability coverage for unexpected toxicity was provided by the U.S. Army, and for malpractice by The Johns Hopkins Medical Institutions.

2.3 Experimental Protocol

2.3.1 Objectives

There were three primary objectives of the study: (1) to assess the relationship between plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition; (2) to determine whether erythrocyte acetylcholinesterase and the contractile response of the iris to light are inhibited in a parallel fashion by pyridostigmine; and (3) to assess the inter-individual variations in the concentration-effect relationships described in (1) and (2) above. In addition, we hoped to determine inter-individual differences in the intravenous clearance of pyridostigmine.

2.3.2 Design

The study was conducted as an open design study. The morning after admission (Day 2), each subject received intravenous pyridostigmine bromide, 6000 micrograms in 24 ml normal saline (250 mcg/ml),

over 8 hours by Harvard pump. Two days later (Day 4) each subject was to receive intravenous pyridostigmine, either 4500 or 9000 micrograms in 24 ml normal saline (187.5 or 375 mcg/ml, respectively), again over 8 hours. The lower dose was to be used if the erythrocyte acetylcholinesterase inhibition with 6000 ug reached 55% at any point (Appendix B, which is Appendix II from the protocol). For each intravenous dose administration (Days 2 and 4), the subjects were fasted except for water for 8 hours before the dose and for 2 hours after the infusion was started, when they were allowed to eat if they desired. The meals given to the subjects were not standardized. Following the initiation of the intravenous pyridostigmine infusion, serial blood specimens were obtained at times specified in the protocol, and non-directed questioning regarding the development of any symptoms was used to monitor for clinical adverse reactions. The outline of the study and the details of how it was to be conducted as initially planned are contained in the Study Flow Chart (Appendix C, which is Appendix V from the protocol). All subjects were screened outside the hospital. Drug administration, sample collection, and post-drug toxicology monitoring were performed in the Drug Development Unit/Clinical Research Center of The Johns Hopkins Hospital.

2.4 Clinical Laboratory Examination

All laboratory examinations except for assay of pyridostigmine were done within The Johns Hopkins Medical Institutions. Hematology and chemistry determinations were performed by the Department of Laboratory Medicine (Clinical Laboratory License number 19-1054). The normal values for these determinations are listed in Appendix D. Erythrocyte acetylcholinesterase assays were to be performed in the research laboratory of

the Division of Clinical Pharmacology (see section 2.6.2). The clinical hematology and chemistry tests were to be performed at screening, upon admission to the hospital, and on the morning following each infusion to monitor for safety. If the results of the laboratory tests performed on the morning of discharge were abnormal, the subjects were to return at weekly intervals to repeat the abnormal tests until the values returned to normal or an alternative explanation for the abnormalities was determined.

2.4.1 Hematology

Routine hematologic determinations, including hematocrit, hemoglobin, red blood cell count, white blood cell count with differential count, and platelet count were done with a Coulter^R counter.

2.4.2 Chemistry

Serum was assayed for sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, glucose, uric acid, calcium, phosphate, total protein, albumin, cholesterol, direct and total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatine kinase.

2.4.3 Electrocardiography

Standard 12-lead electrocardiographic tracings were usually taken shortly before admission to the hospital, and in all cases within 12 months of entry. Electrocardiograms were interpreted by a physician on the staff of The Johns Hopkins Hospital who has formally read electrocardiograms for hospitals for 10 years.

2.4.4 Urine Analysis

Urine analysis was performed in the laboratories of the Division of Clinical Pharmacology. Protein, ketones and bilirubin were measured qualitatively, and pH and specific gravity were quantitatively determined. A microscopic examination of the sediment was also performed.

2.5 Specimen Handling

2.5.1 Blood Collection and Storage

Most blood specimens were obtained by means of a heparin lock inserted prior to drug administration, though occasionally blood was obtained by venipuncture. Specimens were obtained from the arm contralateral to the infusion. Specimens for pyridostigmine concentration and erythrocyte acetylcholinesterase determination were obtained at the times indicated in Appendix E (Appendix III from the protocol) and were handled as described in Section 2.6. Routine chemistry and hematology clinical specimens were placed in appropriate Vacutainer^R tubes and sent to the hospital's Clinical Laboratory for analysis.

2.5.2 Urine Collection

A urine specimen for pyridostigmine assay was collected before each dose of pyridostigmine, from Hour 6 to Hour 8 during each infusion, and for two hours after the infusion was completed.

2.5.3 Specimen Shipment

The frozen plasma and blood specimens were shipped in a sealed insulated container packed with dry ice. Shipment was made by an overnight carrier to Dr. Emil Lin at the University of California at San Francisco.

2.6 Pyridostigmine and Erythrocyte Acetylcholinesterase Determinations

2.6.1 Pyridostigmine Analysis

Blood samples of 5 ml each for pyridostigmine assay were obtained in plastic syringes, placed in heparinized Vacutainer^R tubes, and inverted to insure adequate mixing. The samples were then promptly placed in ice water for transport to our laboratory. As soon as possible (generally within five minutes), the samples were centrifuged for ten minutes in a refrigerated centrifuge. The plasma was separated, transferred to labelled plastic containers, frozen in acetone-dry ice, and stored at -80°C until it was shipped in dry ice to the assay site. The entire process from blood drawing to freezing was less than 20 minutes.

The assay of pyridostigmine in plasma was also performed under Contract DAMD17-86-C-6150, USAMRDC, in the laboratory of Dr. Emil Lin at the School of Pharmacy, University of California at San Francisco (20). The assay utilizes protein precipitation with acetonitrile, pre-column purification on a small C8 Bond Elut^R column, and then high performance liquid chromatography on a silica column with ultraviolet detection. The assay as performed for plasma samples in this study is sensitive to 1.39 ng/ml of pyridostigmine free base. Accuracy is 3 to 12% in the concentration range of 0 to 24 ng/ml. Precision is 6 to 18% (12). For urine, a modified assay was used, which had a minimum quantitation limit of 13.9 ng/ml of pyridostigmine free base, accuracy -8% to 7% in the concentration range 0-7.26 mcg/ml, and precision 2-3% (12).

2.6.2 Erythrocyte Acetylcholinesterase Determinations

Blood samples for erythrocyte acetylcholinesterase determinations (12 ml at baseline and 3 ml each time thereafter) were obtained at the same times as the plasma pyridostigmine samples. The blood was obtained in plastic syringes, transferred into Vacutainer^R tubes containing ethylenediaminetetraacetic acid (EDTA), mixed immediately and brought to the Clinical Pharmacology laboratories for immediate assay -- generally within five minutes of collection. These specimens were kept at ambient temperature until assayed. The assay was performed according to the Standard Operating Procedure (SOP) for the assay of the Analytical Chemistry Branch, USAMRICD, Aberdeen Proving Ground, Maryland 21010 dated 18 June 1985 (21). As performed at Johns Hopkins, the assay is linear between 2.94 and 14.70 uM/ml/min of product produced, with a coefficient of variation determined from the quality control standard less than 2%. Details of the assay and its performance at Johns Hopkins are contained in Appendix F.

2.6.3 Pupillary Response

Pupillary response was measured in the subject's room. External light was excluded with masking tape. A special camera was used to take pictures of the pupils at the times noted in Appendix G (which is Appendix IV from the protocol). The photographic assessment of pupil size is adapted from the work of Ramsay and Woodruff (22).

2.7 Pharmacokinetic and Pharmacodynamic Analyses

2.7.1 Pharmacokinetic Analysis

Visual inspection of the pyridostigmine plasma concentration time-curves following the administration of intravenous

pyridostigmine revealed a rise in the pyridostigmine concentration over the first few hours, followed by a plateau for the remainder of the intravenous administration, and then a decline after the infusion was discontinued. A manual graphic analysis of the decline in concentrations following the end of the infusion was performed. A monophasic decline occurred in most subjects. Macro- and micro-elimination rate constants were estimated and the values used as initial estimates for the curve fitting process. PCNONLIN^R, a commercially available program for the estimation of pharmacokinetic parameters, was used to estimate the variables which best fit the observed data to a one-compartment model (23). Data points were weighted to the reciprocal of the value because this appeared to be the best method of estimating both the high and the low plasma pyridostigmine concentrations. This parameter estimation process produced estimates for the rate constant of elimination, K_{10} ; the intercompartmental rate constants, K_{12} and K_{21} ; and the volume of the central compartment, V . The Nelder-Mead method was used to obtain the best parameter estimates (24).

2.7.2 Pharmacodynamic Modeling

An evaluation of pyridostigmine effect (erythrocyte acetylcholinesterase activity) was conducted for each intravenous dose. The degree and duration of acetylcholinesterase inhibition was determined, and the areas under the inhibition-time curves were compared. Best estimates for various parameters were obtained using a one-compartment model with an effect compartment assuming the E_{\max} model for pharmacodynamic modelling (25-28).

2.8 Statistical Methods

Estimates of the subject population parameters and the mean and

model Effect as
rbc ACh A vs. Inhib.? (plate)
(table)

any sigmoidicity in C-E relationship?

$$rbc AChE Act = baseline - \frac{E_{\max} \cdot C_e}{IC_{50} + C_e}$$

standard deviation of the calculated variables have been determined using standard formulae (29).

3. RESULTS

3.1 Amendments and Compliance

3.1.1 Amendments

The protocol called for six white and six non-white males to be enrolled into the study. Prior to beginning the study, permission was granted to allow Oriental men to enter the study, and the overall intent of this provision in the protocol was interpreted as an effort to prevent a marked imbalance of one racial group in the study, rather than to inflexibly demand that the racial distribution between blacks and whites be perfectly balanced.

The COTR also gave us permission to stop the sequential erythrocyte acetylcholinesterase assays following drug administration once the inhibition from baseline fell to 5% or less prior to the 14th hour after starting the infusion.

3.1.2 Compliance

Only one subject who was enrolled into the study did not fall within 10% of the ideal weights for height as described in the Metropolitan Life Insurance Company tables. This subject (Subject #3) was 1.8 kg "underweight." This subject was allowed to enter the study because his weight was stable and he was completely healthy by all other measures.

There were occasional deviations of actual sample collection from the scheduled sampling times during the course of the project.

These deviations were usually a matter of only minutes, though some of the 24-hour samples were obtained as early as 2.0 hours before or 1.6 hours after the scheduled time. The minor deviations were usually due to difficulties with blood drawing. The earliest specimen collections at 24 hours were due to delays in standardizing the equipment before the dose was given, moving up the "24-hour" sample which was collected the next day approximately on time according to the original schedule. The longest delays at 24 hours were due to problems with standardizing the instrument used to assay acetylcholinesterase first thing in the morning; blood samples were not obtained until the machine was standardized and working properly. All of the deviations of sample collection from the prescribed times can be found in Tables 2-3. All calculations from the results obtained in this study are based on actual times of sample collection, not scheduled times.

Screening laboratory tests were performed more than 14 days before entry into the research unit for six subjects. The interval was 20 days for Subjects #1 and #3, 18 days for Subject #2, 19 days for Subject #6, 21 days for Subject #7, and 15 days for Subject #12. The complete screening history and physical examination were performed 24 days before entry for Subject #8, but were briefly reviewed at the time of his admission.

Two subjects (Subjects #4 and #9) had mechanical failures during the second drug infusion causing incomplete infusions. In two other subjects the acetylcholinesterase assay became non-functional just as the infusions were ending, causing us to lose some data points in the decay curve. Despite the incomplete data for these subjects, it was agreed with the COTR

that these subjects need not be re-dosed or replaced.

The clinical laboratory specimens to assess safety were obtained as specified on the morning following each drug infusion except for Subjects #1 and #2, whose first specimens for safety (after the first infusion) were drawn 24 hours later than specified in the protocol.

3.2 Description of Population of Subjects

Forty-seven different men, 18-35 years of age, were screened for participation in the study once or more using criteria outlined in Section III. A. and B. of the protocol. Individuals were screened more than once if their initial abnormalities seemed minor and might have resolved after a period of days to weeks, enabling them to enter the study if the abnormalities had resolved upon repeat testing. From this pool, twelve subjects who met the laboratory criteria and passed the history and physical examination were chosen to participate in the inpatient study. Reasons for rejection among the 35 individuals assessed who failed the screening evaluation included the following:

- (a) 30 volunteers were rejected for elevated creatine kinase levels;
- (b) 16 volunteers were rejected for low white blood cell counts;
- (c) 14 volunteers were rejected for low hematocrits;
- (d) 13 volunteers were rejected for elevated serum levels of hepatic transferases; and

(e) 2 volunteers failed the history and/or physical examination.

This summary totals more than 35 volunteers because some failed more than one test and some were screened more than once and failed for the same or different reasons.

The twelve volunteers meeting laboratory testing criteria and passing the history and physical examination were admitted to The Johns Hopkins Hospital and entered into the study. Of the twelve who successfully completed the study, seven were white and five were black. The average age was 26 years and ranged from 18 to 35 years. Relevant vital statistics of these volunteers are listed in Table 4.

3.3 Remuneration

Twelve subjects successfully completed the entire project. Eleven were paid \$360.00, while one (Subject #2) received \$375.00, the additional amount for extra samples obtained. One additional payment of \$10 was made to Subject #6, who returned at our request for follow-up laboratory testing after discharge values returned abnormal. One individual received a \$20 finder's fee for successfully recruiting two associates into the study. Four individuals received \$50.00 each for serving as back-ups for the study. For the entire study, \$4,565.00 was distributed to the volunteers.

3.4 Clinical Results

3.4.1 Symptomatic

During the intravenous infusions, two subjects (Subjects #2 and #10) developed symptomatic complaints. Subject #2 complained of nausea, dizziness, weakness, and lethargy during both infusions. With the

first infusion, the symptoms began at about 5 hours into the infusion and resolved after about an hour despite continuing the infusion. With the second infusion, the same symptoms developed at about the same time, but were moderate in severity (compared with mild on the first occasion) and continued until about 20 minutes after the infusion was completed. Subject #10 developed numbness in the left foot starting at the end of the second infusion. This did not immediately resolve after the infusion was discontinued, but had resolved by the next morning.

Subject #1 complained of nausea and dizziness the morning after the second infusion. This was continuing at the time he was discharged from the unit later that same morning. He was instructed to call back if it did not resolve by the next day, and he did not call back, so exactly when these symptoms resolved is uncertain.

After completing the study and leaving the hospital, Subject #11 had an apparent episode of loss of consciousness. The details of this event were transmitted to the COTR, Col. Brian Schuster, and summarized in written form (Appendix H). At his request, this event was reported to the FDA as a possible adverse drug experience (Appendix H). Officials in the U.S. Army Medical Research and Development Command who subsequently reviewed the details in the summary agreed with our assessment that the incident did not appear to be related to the administration of intravenous pyridostigmine which had ended approximately 18 hours before this incident occurred (Appendix H).

3.4.2 Vital Signs

None of the subjects had a clinically significant change

in temperature, blood pressure, heart rate, or respiratory rate following the administration of any of the pyridostigmine bromide doses.

3.4.3 Laboratory

3.4.3.1 Liver Function Tests

Three subjects developed slight abnormalities of liver function tests during the course of the study. These subjects were Subjects #4, #6, and #12. In each case the alanine aminotransferase level was above the upper limit of normal (30 units/liter) only on the day of discharge. The elevations were 33-34 units/liter. On the same dates the aspartate aminotransferase levels were still normal in all three subjects. None of these subjects had any symptoms of hepatic dysfunction and were discharged as scheduled per protocol. Subject #6 returned two weeks later, and his follow-up values were normal.

3.4.3.2 Creatine Kinase

One volunteer (Subject #1) had a normal creatine kinase level at screening but an elevated level (241 units/l) at the time of admission (normal = 0-160 units/l). His next creatine kinase level, just prior to administration of the second intravenous dose of drug, was normal at 128 units/l. The next morning it was slightly elevated at 184 units/l. Another volunteer (Subject #9) had a slightly elevated creatine kinase at screening (183 units/l), but it had been normal 21 days earlier, it was normal on admission, and it remained normal throughout the study period. The creatine kinase did not increase above the normal range in any of the other subjects during the study.

3.4.3.3 Other Clinical Chemistry Tests

No other clinical chemistry tests showed any significant changes over the duration of the protocol.

3.4.3.4 Hematological Testing

All subjects had normal hematological values at the time of admission. Subject #11 was slightly anemic at screening (hemoglobin = 13.6 g/dl) but normal at the time of admission (14.2 g/dl). None of the subjects had a fall of hematocrit greater than 5% from their baseline values.

3.4.3.5 Electrocardiograms

All subjects were found to have electrocardiograms free of any evidence of clinically significant abnormalities. Repeat electrocardiograms were not obtained, which was in accordance with the specifications of the protocol.

3.4.4 Clinical Conclusions

The subjects tolerated their hospitalization with frequent blood drawing and the administration of the test drug fairly well. The only symptoms that occurred during the infusion that resolved while the infusion continued or shortly after the infusion ended were nausea, dizziness, weakness, and lethargy in Subject #2. The minimal elevations of ALT noted in three subjects on the morning following the second infusion were not clinically significant. The adverse events observed in this study are summarized in Table 5, and the criteria used to categorize their relationship to the drug are provided in Appendix I.

3.5 Pharmacokinetics and Pharmacodynamics

3.5.1 Pharmacokinetics

The concentration of pyridostigmine expressed in nanograms of base per ml of plasma is given in Tables 6-7 (12). The intravenous administrations produced rising concentrations for the first few hours, followed by a plateau for the remainder of the infusion, and then falling concentrations after the administration was stopped after eight hours.

The areas under the pyridostigmine concentration-time curve (AUC) were calculated for both intravenous infusions from the best fit parameter estimates fitting the plasma-concentration data to a one-compartment model. The calculated areas under the concentration-time curve are displayed in Table 8. Because the curve-fitting for the two infusions was conducted simultaneously, the mean area under the pyridostigmine concentration-time curve after the second intravenous infusion (134.45 ng·hr/ml) was 50% higher than the mean area after the first infusion (89.99 ng·hr/ml). Since the ~~observed~~ estimated plasma concentrations for both infusions were well described by the same best-fit estimates, this implies linear pharmacokinetics across this dosing range (Figures 2-11). There was less than a twofold difference between the maximum and minimum pyridostigmine AUC's with each intravenous dose and the coefficient of variation of the areas was only about 16%.

Table 8 also summarizes the volume of the single compartment, the elimination rate constant, and the plasma clearance of pyridostigmine base. The mean elimination rate constant was 1.365 hr^{-1} , which corresponds to a half-life of 0.5 hours. The mean plasma clearance was 44.62

liters/hr, or 744 ml/minute.

Two subjects had very low concentrations of pyridostigmine measured in their urine specimens collected before infusions (Table 9). The concentrations were 0.0181 mcg/ml before the second infusion for Subject #6, and 0.0174 mcg/ml before the first infusion for Subject #10. We presume these trivial levels were induced by foods, were random positive assays from a very sensitive (and thus not totally specific) assay, or reflected residual pyridostigmine from the first infusion in the case of Subject #6.

*was rbc ACE Act. baseline @ start
7 2nd inf.?*

The results of the urine collections at "steady state," Hours 6-8 during the infusion, are shown in Table 9. Perhaps the most noteworthy observation is the vast difference in measured urinary volume over the course of the two hours for the different subjects, ranging from 13-fold with the first infusion to nearly 65-fold with the second infusion! The very low urinary volumes for Subject #11 are especially suggestive of incomplete urination at the end of the two hours. Even without considering that subject's urinary volumes, there was still a sixfold difference in urinary volume during the first infusion and nearly a tenfold range in volume between subjects with the second infusion. These small, probably incomplete urine collections are largely responsible for the wide variation in calculated total renal excretion of pyridostigmine base during the final two hours of the infusion, the presumed "steady state" period. Because of the unreliable urinary collections, the renal clearances calculated in Table 10 are also unreliable.

3.4.2 Pharmacodynamics

baseline rbc AChE Act?
variability?

The degrees of inhibition of erythrocyte acetylcholinesterase activity after the two doses of intravenous pyridostigmine bromide are shown in Tables 11-12. These data reveal that inhibition increased over the first 3-5 hours and then plateaued until the infusion was stopped at 8 hours, whereupon the inhibition steadily fell over the next 4-6 hours. The time course of inhibition was similar with both infusions (Figures 2-11), but the magnitude of the inhibition was in all cases greater with the higher dose. The average degree of erythrocyte acetylcholinesterase inhibition for the 10 subjects who received both doses is shown in Figure 12.

Table 13 shows the maximal erythrocyte acetylcholinesterase inhibition with the two infusions. Except for Subject #11, the maximal inhibition was greater with the second infusion than with the first. Nonetheless, in many cases the higher maximal inhibition with the second infusion was only slightly greater than with the first infusion and for Subject #11 it was the same for both infusions. These intravenous infusions were excellent in achieving a range of 20-40% inhibition, exceeding 40% in no subjects.

Table 8 summarizes the pharmacodynamic constants as well as the pharmacokinetic constants. Of particular note is the IC_{50} , the concentration of pyridostigmine base which produces 50% inhibition of the erythrocyte acetylcholinesterase. The mean IC_{50} was 31.82 ng/ml with a coefficient of variation of only 11.8%.

Unfortunately, our efforts to photograph and measure the pupil size in these subjects during the intravenous infusions were unrewarding. Despite substantial efforts to standardize and objectify pupil measurements, we were never convinced that we were reliably and reproducibly doing so. Therefore, we present no data regarding pupil size or the influence of the infusions on the contractile response of the iris.

4. DISCUSSION

These data demonstrate that the infusion of 6000 or 9000 mcg/ml of pyridostigmine bromide at a constant rate over eight hours produces a "steady state" with regard to pyridostigmine base concentrations and erythrocyte acetylcholinesterase inhibition. Such doses of pyridostigmine were well tolerated, not associated with significant adverse events.

The mean elimination rate constant was 1.365 hr^{-1} . This is a lower value than seen in our previous studies, where the mean rate constants were 2.343 hr^{-1} (30) and 3.307 hr^{-1} (9). This difference is most likely due to the method of analysis. A two-compartment analysis was used to analyze the prior studies. In this study, a single-compartment model was sufficient to model the data. More frequent sampling of plasma at the onset and offset of the infusion would have been required in order to conduct a two-compartment analysis. Nonetheless, the mean plasma clearance, a model-independent parameter, was remarkably similar in both studies (744 ml/minute in this study, 779 ml/minute in our earlier study) (9). The coefficient of variation of the plasma clearance was only 15.60%, suggesting quite uniform pharmacokinetics of intravenous pyridostigmine in healthy volunteers

going to a 2-com
results in a smaller $t_{1/2}$

[correcting the clearance for body weight]?

Our observations allowed us to assess the influence of various plasma pyridostigmine base concentrations on acetylcholinesterase activity, and calculate a concentration at which 50% of the erythrocyte acetylcholinesterase activity would be inhibited (IC_{50}). The IC_{50} for this group of subjects was 31.82 ± 3.72 ng/ml (mean \pm standard deviation), which is in close agreement with our calculated value of 29.46 ± 6.84 ng/ml in an earlier study (9). The small coefficient of variation (11.76%) of the IC_{50} estimate in this study suggests that the IC_{50} may actually be identical in all subjects and that the variability of the estimate results from experimental error in determining the pyridostigmine concentrations, the acetylcholinesterase activity, and the mathematical modelling process. The sensitivity of AChE in all subjects is likely to be identical. Molecular heterogeneity of acetylcholinesterase is unlikely to be a factor. The greater variability in IC_{50} in our earlier study which suggested sensitivity differences must have resulted from the errors generated by modelling a less optimal data set.

differences
in
C \rightarrow C_e
Keo

but had more data
for 2-com? ... less optimal
PA data?

None of our subjects exceeded 40% inhibition of erythrocyte acetylcholinesterase inhibition. In fact, the maximal and "steady state" inhibitions of erythrocyte acetylcholinesterase were within the reputed target range for acetylcholinesterase inhibition which will provide significant protection from organophosphate poisoning without causing serious adverse events.

5. CONCLUSIONS

These data provide guidance for the plasma pyridostigmine base

concentrations that need to be achieved and maintained in order to reach a target range of 20-40% inhibition of erythrocyte acetylcholinesterase activity. We also demonstrated that the pharmacokinetics of pyridostigmine are linear over the range studied, that the elimination half-life is 30 minutes, and the IC_{50} is 31.8 mcg/ml. Our efforts to assess a pharmacodynamic response outside the vascular compartment (i.e., in the contractile response of the iris) were unsuccessful because of technical difficulties in measuring pupil size from photographs. Intersubject variations in the response to oral pyridostigmine are likely to result from differences in pyridostigmine concentrations due to intersubject differences in the extent of pyridostigmine absorption and rate of pyridostigmine systemic clearance. Differences in intersubject sensitivity of the acetylcholinesterase enzyme to pyridostigmine are unlikely to be important.

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TABLE 1

Amount of Pyridostigmine Base Administered to Each Subject

<u>Subject</u>	First Infusion		Second Infusion	
	<u>Concentration in Aliquot</u> (mcg/ml)	<u>Total Dose Infused *</u> (mcg)	<u>Concentration in Aliquot</u> (mcg/ml)	<u>Total Dose Infused #</u> (mg)
1	162.28	3894.72	163.92	5901.12
2	162.46	3899.04	161.46	5812.56
3	156.56	3757.44	161.46	5812.56
4	159.50	3828.00	159.66	@
5	180.16	4323.84	170.38	6133.68
6	164.72	3953.28	167.32	6023.52
7	168.08	4033.92	166.26	5985.36
8	145.46	3491.04	167.94	6045.84
9	133.20	3196.80	158.64	@
10	166.98	4007.52	154.70	5569.20
11	163.76	3930.24	159.24	5732.64
12	167.56	4021.44	156.16	5621.76
MEAN	160.89	3861.44	162.26	5863.82
SD	11.90	285.57	4.93	187.23
CV (%)	7.40	7.40	3.04	3.19
MAX	180.16	4323.84	170.38	6133.68
MIN	133.20	3196.80	154.70	5569.20

* Calculated by multiplying the aliquot concentration by the pump speed (0.05 ml/min) and the time (480 minutes).

Calculated by multiplying the aliquot concentration by the pump speed (0.075 ml/min) and the time (480 minutes).

@ Pump malfunction prevented entire dose from being administered.

TABLE 2
Sampling Times During First Infusion of Pyridostigmine

Subj #	SCHEDULE TIME OF SAMPLE											
	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
	8.50	9.00	9.50	10.00	11.00	12.00	14.00	24.00	ACTUAL TIME OF SAMPLE (HOURS)			
1	0.00	0.25	0.50	1.00	1.62	2.00	3.00	4.00	5.00	6.02	7.00	8.00
2	0.00	0.25	0.63	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
3	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
4	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.08	5.00	6.00	7.00	8.00
5	0.00	0.25	0.50	1.00	1.50	2.00	3.10	4.08	5.00	6.00	7.00	8.00
6	0.00	0.25	0.52	1.00	1.50	2.00	3.00	4.00	5.02	6.00	7.00	8.00
7	0.00	0.25	0.50	1.00	1.50	2.02	3.00	4.00	5.00	6.00	7.00	8.00
8	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
9	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
10	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00
11	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.02	8.00
12	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00

TABLE 3
Sampling Times During Second Infusion of Pyridostigmine

Subj #	SCHEDULE TIME OF SAMPLE												ACTUAL TIME OF SAMPLE (HOURS)											
	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.00	11.00	12.00	14.00	24.00			
1	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.02	8.00	8.25	8.50	9.03	9.47	10.00	11.00	12.00	14.00	23.98			
2	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.52	10.00	11.00	12.00	14.00	24.00			
3	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.00	11.00	12.75	14.00	24.03			
4	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.12	8.02	8.42	8.67	9.17	9.67	10.17	11.17	12.17	13.90	24.00			
5	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.02	8.25	8.50	9.00	9.50	10.00	11.00	12.00	14.00	24.00			
6	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.00	11.00	12.00	14.00	24.00			
7	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.03	9.50	10.00	11.00	12.00	14.00	24.00			
8	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.00	11.00	12.00	14.00	22.33			
9	0.00	0.25	0.50	1.00	1.50	2.00	3.00	3.88	5.00	6.00	7.00	8.00	*	*	9.00	*	10.00	11.00	12.00	14.00	22.62			
10	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.03	11.00	12.00	14.00	22.00			
11	0.00	0.25	0.53	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.27	8.50	9.00	9.52	10.00	11.00	12.00	14.00	24.03			
12	0.00	0.25	0.50	1.00	1.50	2.00	3.00	4.00	5.00	6.00	7.00	8.00	8.25	8.50	9.00	9.50	10.00	11.00	12.00	14.00	24.12			

* Sample not obtained

TABLE 4
Vital Statistics of Subjects

<u>Patient #</u>	<u>Race</u>	<u>Age</u>	<u>Height (cm)</u>	<u>Weight (kg)</u>
01	C	30	173	71.0
02	C	31	175	69.0
03	B	24	180	58.0
04	C	27	172	81.5
05	C	32	182	88.0
06	B	35	180	73.2
07	C	23	182	86.5
08	C	24	178	75.0
09	C	23	182	73.0
10	B	26	185	70.5
11	B	21	173	67.0
12	B	18	178	69.0
<hr/>				
AVERAGE \pm S.D.		26 \pm 5	178 \pm 4	73.5 \pm 8.9
RANGE		18-35	172 - 185	67.0 - 88.0

TABLE 5
Summary of Adverse Events

<u>Subject #</u>	<u>Adverse Events</u>	<u>Relationship to Study Drug *</u>
1	Nausea, dizziness starting morning after second dose	Possible
	Minimal CPK elevation morning after second dose.	Possible
2	Nausea, dizziness, weakness, lethargy at 5 hours into first dose, resolved after one hour despite continued infusion.	Possible
	Nausea, dizziness, weakness, lethargy during latter portion of second dose, resolved 20 minutes after infusion complete.	Possible
3	None	
4	ALT increased to 34 units/L on morning after second dose.	Possible
5	None	
6	ALT increased to 33 units/L on morning after second dose.	Possible
7	None	
8	None	
9	None	
10	Numbness in left foot starting at the end of the second infusion, resolved by next morning.	Possible
11	None during study; loss of consciousness afternoon following discharge (see Appendix H).	Definitely Not
12	ALT increased to 33 units/L on morning after second infusion.	Possible

* For definitions of terms used in this column, see Appendix G.

Plasma Concentrations of Pyridostigmine During and Following an Eight Hour Infusion
6000 ug Infusion of Pyridostigmine Bromide

	SCHEDULED TIME OF SAMPLE (hours after dose)											
	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
SUBJ#				PYRIDOSTIGMINE CONCENTRATION ng base/ml								
1	*	*	2.62	6.82	9.03	8.43	9.75	10.30	10.70	9.47 ¹	10.90	12.60
2	*	3.96	6.31	9.17	10.30	9.71	10.50	12.30	12.30	12.00	12.00	12.10
3	*	8.27	11.90	11.60	14.60	17.00	12.90	15.60	14.50	14.20	15.60	16.40
4	*	3.63	6.21	5.89	7.02	8.44	8.35	8.64	10.50	9.08	9.33	10.20
5	*	3.41	4.55	9.07	8.47	11.60	10.00	10.80	13.60	12.30	12.90	13.20
6	*	3.88	3.73	6.32	6.38	8.56	11.80	12.70	13.70	11.10	12.60	14.90
7	*	5.64	4.26	7.57	7.02	8.53	9.66	10.70	11.50	11.60	12.20	11.00
8	*	6.56	5.82	11.90	9.19	8.85	10.00	11.50	10.30	11.40	11.10	9.81
9	*	4.54	6.93	7.85	9.40	8.79	12.70	9.57	10.40	10.10	8.86	9.57
10	*	6.23	7.28	9.91	8.28	11.20	11.80	11.30	12.50	11.70	13.20	12.30
11	*	3.22	5.56	10.20	10.30	11.90	11.80	9.70	10.10	11.50	13.10 ²	11.60
12	*	3.75	5.45	6.73	8.45	9.07	10.30	9.32	9.78	10.50		9.45
# ⁴	0	11	12	12	12	12	12	12	12	11	11	12
MEAN		4.83	5.89	8.59	9.04	10.17	10.80	11.04	11.66	11.41	11.98	11.93
SD		1.62	2.33	2.03	2.15	2.51	1.38	1.88	1.62	1.31	1.89	2.16
CV(%)		33.67	39.53	23.70	23.76	24.64	12.81	17.02	13.88	11.47	15.80	18.09
MAX		8.27	11.90	11.90	14.60	17.00	12.90	15.60	14.50	14.20	15.60	16.40
MIN		3.22	2.62	5.89	6.38	8.43	8.35	8.64	9.78	9.08	8.86	9.45

SUBJ#	SCHEDULED TIME OF SAMPLE (hours after dose)								
	8.25	8.5	9	9.5	10	11	12	14	24
	PYRIDOSTIGMINE CONCENTRATION ng base/ml								
1	8.43	5.96	4.44	3.56	2.57	1.68	*	*	*
2	6.88	5.50	3.28	3.09	2.31	*	*	*	*
3	10.60	8.93	5.76	4.94	3.91	3.78	2.32	*	*
4	5.99	5.08	3.61	3.51	2.88	2.01	*	*	*
5	10.40	9.39	5.62	4.79	3.69	3.19	*	*	*
6	6.23	5.66	3.96	2.96	3.16	1.92	1.69	*	*
7	5.36	5.36	2.72	3.00	*	*	*	*	*
8	5.64	4.22	3.03	2.07	1.96	*	*	*	*
9	4.30	3.67	1.41	*	*	*	*	*	*
10	6.01	4.63	2.68	2.68	2.48	*	*	*	*
11	8.34	81.80 ³	3.27	2.91	2.58	*	*	*	*
12	6.05	3.68	3.14	2.86	1.96	*	*	*	*
# ⁴	12	11	12	11	10	5	2	0	0
MEAN	7.02	5.64	3.58	3.31	2.75	2.52	2.01		
SD	1.99	1.90	1.23	0.87	0.67	0.92	0.45		
CV(%)	28.39	33.69	34.49	26.22	24.21	36.44	22.22		
MAX	10.60	9.39	5.76	4.94	3.91	3.78	2.32		
MIN	4.30	3.67	1.41	2.07	1.96	1.68	1.69		

* Concentration less than the detection limit of 1.39 ng base/ml

1 Concentration not included in the statistics. Infusion had been discontinued for a few minutes when the needle was pulled out inadvertently.

2 No sample assayed

3 Concentration not included in the statistics; contamination suspected.

4 Number of samples with concentrations above the minimum detectable limit

Plasma Concentrations of Pyridostigmine During and Following an Eight Hour Infusion
9000 ug Infusion of Pyridostigmine Bromide

		SCHEDULED TIME OF SAMPLE (hours after dose)										
	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
SUBJ#		PYRIDOSTIGMINE CONCENTRATION ng base/ml										
1	*	2.74	6.89	8.86	13.20	13.50	20.40	17.90	20.00	20.50	20.20	20.60
2	*	6.63	9.28	13.00	13.80	13.90	15.60	18.20	16.40	16.60	18.00	18.40
3	*	6.00	11.40	19.20	22.80	20.20	23.80	19.80	20.60	18.40	23.10	22.40
4	*	5.21	6.87	8.67	12.70	12.50	12.70	14.00	12.60	12.80	8.30 ¹	6.55 ¹
5	*	6.52	²	10.50	14.90	14.10	18.40	14.40	14.40	15.50	19.40	19.60
6	*	8.82	10.60	10.90	14.30	16.80	16.70	19.10	17.40	18.00	16.50 ²	17.40
7	*	6.13	11.70	12.40	22.40	11.00	15.50	15.10	14.30	15.20		14.50
8	*	6.76	11.00	11.80	12.60	13.90	15.10	17.80	16.40	14.50	14.70	15.30
9	*	5.52	6.65	10.00	9.83	10.50	11.70	4.55 ¹	2.12 ¹	*	*	*
10	*	8.61	10.70	11.40	11.80	16.50	14.40	16.20	19.00	17.80	20.00	16.10
11	*	2.05	3.52	6.04	9.95	18.10	12.40	8.08	6.77	10.40	13.10	8.11
12	*	6.55	9.45	12.60	14.50	15.60	15.30	12.90	11.30	16.00	13.30	13.40
# ⁴	0	12	11	12	12	12	12	11	11	11	9	10
MEAN		5.96	8.91	11.28	14.40	14.72	16.00	15.59	15.38	15.97	17.59	16.58
SD		1.99	2.59	3.18	4.16	2.85	3.48	3.30	4.10	2.80	3.44	4.10
CV(%)		33.32	29.09	28.22	28.88	19.38	21.74	21.16	26.69	17.52	19.54	24.72
MAX		8.82	11.70	19.20	22.80	20.20	23.80	19.80	20.60	20.50	23.10	22.40
MIN		2.05	3.52	6.04	9.83	10.50	11.70	8.08	6.77	10.40	13.10	8.11

SUBJ#	SCHEDULED TIME OF SAMPLE (hours after dose)								
	8.25	8.5	9	9.5	10	11	12	14	24
	PYRIDOSTIGMINE CONCENTRATION ng base/ml								
1	11.50	9.20	7.78	6.65	4.08	2.74	2.61	*	*
2	12.80	10.00	8.12	4.90	5.15	*	*	*	*
3	12.10	9.72	7.90	6.00	6.08	3.65	1.67	*	*
4	5.92 ¹	4.71 ¹	4.22 ¹	3.86 ¹	1.96 ¹	*	*	*	*
5	13.20	9.69	11.30	3.47	3.20	1.82	*	2.03	*
6	8.50	7.36	4.97	2.96	3.04	*	1.95	1.46	*
7	7.43	5.36	3.91	3.80	3.00	*	*	*	*
8	9.07	7.11	6.12	3.51	2.87	2.57	*	*	*
9	2	2	*	2	*	*	*	*	*
10	9.56	7.88	3.78	3.73	3.48	1.98	1.63	*	*
11	4.35	3.57	2.72	2.17	*	*	*	*	*
12	8.61	4.25	3.77	2.97	2.66	3	*	*	*
# ⁴	10	10	10	10	9	5	4	2	0
MEAN	9.71	7.41	6.04	4.02	3.73	2.55	1.97	1.75	
SD	2.74	2.35	2.70	1.41	1.17	0.73	0.45	0.40	
CV(%)	28.24	31.69	44.67	35.17	31.30	28.42	23.05	23.10	
MAX	13.20	10.00	11.30	6.65	6.08	3.65	2.61	2.03	
MIN	4.35	3.57	2.72	2.17	2.66	1.82	1.63	1.46	

* Concentration less than the detection limit of 1.39 ng base/ml

1 Concentration not included in the statistics. Infusion terminated early due to technical difficulties

2 No sample assayed

3 Bad chromatogram, insufficient sample to repeat, no value obtained

4 Number of samples with concentrations above the minimum detectable limit

TABLE 8

BEST ESTIMATES OF PHARMACOKINETIC AND PHARMACODYNAMIC
CONSTANTS FOR A ONE-COMPARTMENT MODEL WITH AN EFFECT COMPARTMENT
ASSUMING THE F_{MAX} MODEL FOR PHARMACODYNAMIC MODELLING

(Estimates obtained by Simultaneously Fitting Data from Both Infusions)

Subject	V ± SEM	K ₁₀	K _{EO}	IC ₅₀	AUC-A	AUC-B	CL
1	48.44 ± 2.55	0.829 ± 0.014	2.52 ± 1.35	32.54 ± 3.14	97.03 ± 1.79	147.01 ± 2.72	40.14 ± .74
2	38.26 ± 1.87	1.089 ± 0.054	1.95 ± 0.77	37.47 ± 3.17	93.62 ± 1.46	139.57 ± 2.18	41.65 ± .65
3	22.42 ± 1.69	1.425 ± 0.109	1.24 ± 0.51	32.98 ± 4.60	117.57 ± 2.61	181.88 ± 4.03	31.96 ± .71
4*							
5	50.39 ± 2.90	0.852 ± 0.049	**9.647 ± 18.00	36.10 ± 4.37	100.66 ± 1.84	142.80 ± 2.76	42.95 ± .83
6	33.47 ± 2.44	1.298 ± 0.090	1.617 ± 0.68	39.30 ± 3.87	90.99 ± 2.01	138.64 ± 3.07	43.45 ± .96
7	29.79 ± 2.42	1.689 ± 0.138	1.647 ± 0.529	31.57 ± 2.87	80.18 ± 1.88	118.97 ± 2.79	50.31 ± 1.18
8	29.02 ± 2.36	1.596 ± 0.131	2.047 ± 0.938	30.79 ± 3.56	75.36 ± 1.73	130.52 ± 3.00	46.32 ± 1.07
9*							
10	26.95 ± 1.87	1.549 ± 0.109	1.382 ± 0.48	29.37 ± 3.19	96.00 ± 1.90	133.41 ± 2.64	41.74 ± 0.83
11	37.81 ± 6.58	1.524 ± 0.271	1.732 ± 1.28	28.11 ± 5.68	68.18 ± 3.34	99.45 ± 4.87	57.64 ± 2.83
12	27.77 ± 1.93	1.803 ± 0.127	1.445 ± 0.39	24.97 ± 2.27	80.33 ± 1.55	112.29 ± 2.16	50.06 ± 0.97
MEAN	34.43	1.365	1.73	31.82	89.99	134.45	44.62
STD DEV	9.27	0.341	0.39	3.74	13.61	21.20	6.96
CV (%)	26.92	24.945	22.68	11.76	15.13	15.77	15.60
MAX	50.39	1.803	2.52	37.47	117.57	181.88	57.64
MIN	22.42	0.829	1.24	24.97	68.18	99.45	31.96

** excluded from group statistics
because he was outlier

t/2 K_{EO} = 25 min

* = Subjects excluded from analysis because they only received one complete infusion
V = Volume of compartment (L)
K₁₀ = Rate constant of pyridostigmine from the compartment (hr⁻¹)
K_{EO} = Rate constant of elimination of pyridostigmine from effect compartment (hr⁻¹)
IC₅₀ = Concentration of pyridostigmine base in ng/ml producing 50% inhibition of RBC acetylcholinesterase (ng/ml)
AUC-A = Area under concentration-time curve for the 6000 mcg infusion (ng·hr/ml)
AUC-B = Area under concentration-time curve for the 9000 mcg infusion (ng·hr/ml)
CL = Plasma clearance of pyridostigmine base (L/hour)

correct for
Clearance/body weight

L/hr

~~mg/min~~

Cl (L/hr/kg)

	Cl	wt (kg)	
1	40.1	71.0	~ 565
2	41.6	69.0	604
3	32.0	58.0	551
4	—	81.5	—
5	43.0	88.0	488
6	43.4	73.2	594
7	50.3	86.5	582
8	46.3	78.0	618
9	—	73.0	—
10	41.7	67.0 70.5	593
11	52.6	69.0	861
12	50.1	69.0	726

488 - 726

7
306
323
488

Max - 861

min - 488

max/min = ~ 1.765

Mean

S.D.

C.V.



TABLE 9

Urinary Pyridostigmine Base Excretion
at Steady State (Hours 6-8 of Infusion)

Subject #	Infusion 1 (750 mcg/hr)			Infusion 2 (1125 mcg/hr)		
	<u>Concentration</u> (mcg/ml)	<u>Volume</u> (ml)	<u>Total Excreted</u> (mcg)	<u>Concentration</u> (mcg/ml)	<u>Volume</u> (ml)	<u>Total Excreted</u> (mcg)
1	2.38	247	588	4.26	703	2995
2	5.46	703	3838	3.17	300	951
3	10.3	490	5047	5.36	214	1147
4	3.37	232	782	*	*	*
5	2.99	743	2222	3.29	840	2764
6	6.51	246	1601	10.2	224	2285
7	7.53	182	1370	7.26	274	1989
8	1.33	347	462	2.72	85	231
9	2.03	346	702	*	*	*
10	1.92	539	1035	3.20	307	982
11	12.5	56	700	3.46	13	45
12	2.19	120	263	15.9	136	2162
Mean	4.13	354	1550	5.88	310	1555
S.D.	3.20	212	1415	4.01	249	976
Range	1.33-12.5	56-743	263-5047	2.72-15.9	13-840	45-2995

* Infusion terminated early due to technical difficulties

TABLE 10

Renal Pyridostigmine Base Clearance at Steady State
(Hours 6-8 of Infusion)

<u>Subject #</u>	<u>Avg. Pl. Conc. #</u>	<u>Clearance* for Infusion 1 (750 mcg/hr)</u>	<u>Avg. Pl. Conc. #</u>	<u>Clearance for Infusion 2 (1125 mcg/hr)</u>
1	11.8	24.9	20.4	73.4
2	12.0	160	17.7	26.9
3	15.4	164	21.3	26.9
4	9.54	41.0	x	**
5	12.8	86.8	18.2	75.9
6	12.9	62.1	17.3	66.0
7	11.6	59.1	14.9	66.8
8	10.8	21.4	14.8	7.81
9	9.51	36.9	x	**
10	12.4	41.7	18.0	27.3
11	12.1	28.9	10.5	2.14
12	9.98	13.2	14.2	7.61
Mean	11.7	61.7	16.7	38.1
S.D.	1.59	4.83	3.03	27.9
Range	9.54-15.4	13.2-164	10.5-21.3	2.14-75.9

* Clearance = $\frac{\text{Urine Concentration (mcg/ml)} \times \text{Vol (ml)}}{\text{Avg. Pl. Conc. (ng/ml)} \times 2 \text{ hours}}$
(L/hr)

Avg. Pl. Conc. - Average plasma concentration of pyridostigmine base for Hours 6, 7, and 8 (ng/ml)

TABLE 11

Inhibition of Erythrocyte Acetylcholinesterase During and Following an Eight-Hour
Infusion of 6000 ug Pyridostigmine Bromide

SUBJ#	SCHEDULED TIME OF SAMPLE (hours after dose)											
	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
	INHIBITION OF RED BLOOD CELL ACETYLCHOLINESTERASE (fraction inhibited)											
1	0.00	0.00	0.03	0.15	0.21	0.25	0.28	0.25	0.28	0.18	0.26	0.28
2	0.00	0.00	0.05	0.11	0.17	0.23	0.24	0.26	0.22	0.23	0.25	0.34
3	0.00	0.05	0.08	0.16	0.23	0.28	0.31	0.27	0.27	0.28	0.31	0.38
4	0.00	0.00	0.03	0.10	0.16	0.21	0.23	0.22	0.25	0.22	0.24	0.28
5	0.00	0.01	0.06	0.10	0.18	0.25	0.27	0.25	0.28	0.23	0.26	0.29
6	0.00	0.00	0.01	0.12	0.15	0.27	0.25	0.24	0.24	0.26	0.22	0.27
7	0.00	0.00	0.04	0.09	0.11	0.25	0.25	0.22	0.21	0.24	0.21	0.25
8	0.00	0.05	0.09	0.13	0.19	0.22	0.26	0.27	0.25	0.25	0.28	0.30
9	0.01	0.03	0.07	0.13	0.17	0.24	0.25	0.22	0.22	0.22	0.21	0.27
10	0.01	0.03	0.08	0.18	0.21	0.27	0.28	0.29	0.29	0.28	0.28	0.31
11	0.00	0.03	0.07	0.15	0.20	0.25	0.28	0.28	0.31	0.29	0.32	*
12	0.00	0.05	0.07	0.15	0.19	0.23	0.25	0.29	0.32	0.31	0.30	*
#	12	12	12	12	12	12	12	12	12	12	12	10
MEAN	0.00	0.02	0.06	0.13	0.18	0.24	0.26	0.25	0.26	0.25	0.26	0.30
SD	0.00	0.02	0.03	0.03	0.03	0.02	0.02	0.03	0.04	0.04	0.04	0.04
CV (%)	177.12	106.17	46.57	21.54	17.84	8.22	8.85	10.17	14.00	14.80	14.88	12.25
MAX	0.01	0.05	0.09	0.18	0.23	0.28	0.31	0.29	0.32	0.31	0.32	0.38
MIN	0.00	0.00	0.01	0.09	0.11	0.21	0.23	0.22	0.21	0.18	0.21	0.25

SUBJ#	SCHEDULED TIME OF SAMPLE (hours after dose)								
	8.25	8.5	9	9.5	10	11	12	14	24
	INHIBITION OF RED BLOOD CELL ACETYLCHOLINESTERASE (fraction inhibited)								
1	0.27	0.24	0.15	0.16	0.09	0.02	0.09	*	0.01
2	0.20	0.19	0.12	0.07	0.05	0.07	0.09	*	0.02
3	0.33	0.30	0.23	0.21	0.15	0.10	0.07	0.04	-0.05
4	0.24	0.21	0.16	0.14	0.13	0.11	0.05	0.00	-0.03
5	0.27	0.22	0.15	0.12	0.10	0.11	0.07	0.03	-0.02
6	0.23	0.21	0.16	0.12	0.14	0.09	0.03	*	-0.03
7	0.22	0.16	0.13	0.08	0.09	0.07	0.00	*	-0.01
8	0.26	0.22	0.17	0.11	0.09	0.12	0.13	0.08	0.06
9	0.22	0.18	0.11	0.09	0.05	0.07	*	0.05	0.02
10	0.25	0.20	0.17	0.13	0.10	0.07	0.02	*	-0.01
11	*	*	0.13	0.09	0.06	0.00	0.05	0.00	0.02
12	*	*	0.10	0.08	0.02	0.00	0.07	0.00	-0.02
#	10	10	12	12	12	12	11	7	12
MEAN	0.25	0.21	0.15	0.12	0.09	0.07	0.06	0.03	0.00
SD	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03
CV (%)	15.05	18.09	23.82	34.18	44.06	58.69	60.42	104.92	968.61
MAX	0.33	0.30	0.23	0.21	0.15	0.12	0.13	0.08	0.06
MIN	0.20	0.16	0.10	0.07	0.02	0.00	0.00	0.00	-0.05

* No result, due to technical problems or no sample obtained

TABLE 12

Inhibition of Erythrocyte Acetylcholinesterase During and Following an Eight-Hour
Infusion of 9000 ug Pyridostigmine Bromide

SUBJ#	SCHEDULED TIME OF SAMPLE (hours after dose)											
	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
1	0.00	0.00	0.08	0.19	0.17	0.25	0.32	0.38	0.38	0.36	0.38	0.38
2	0.00	0.01	0.09	*	0.20	0.22	0.24	0.33	0.33	0.32	0.34	0.36
3	0.00	0.04	0.14	0.27	0.34	0.36	0.40	0.37	0.38	0.36	0.39	0.39
4	0.00	0.08	0.14	0.22	0.27	*	0.24	0.34	0.32	0.31	0.19 ¹	0.13 ¹
5	0.02	0.07	0.15	0.22	0.28	0.29	0.27	0.34	0.33	0.34	0.32	0.32
6	0.01	0.07	0.13	0.22	0.27	0.29	0.30	0.33	0.32	0.30	0.32	0.36
7	0.01	0.06	0.15	0.23	0.30	0.30	0.32	0.32	0.33	0.32	0.32	0.35
8	0.00	0.08	0.15	0.23	0.26	0.28	0.34	0.34	0.32	0.32	0.32	0.31
9	0.02	0.08	0.15	0.22	0.26	0.29	0.30	0.18 ¹	0.08 ¹	0.03 ¹	0.02 ¹	0.00 ¹
10	0.00	0.04	0.08	0.20	0.27	0.33	0.34	0.35	0.37	0.38	0.38	0.38
11	0.00	0.00	0.04	0.10	0.20	0.29	0.29	0.25	0.25	0.25	0.32	0.27
12	0.00	0.02	0.11	0.23	0.27	0.35	0.38	0.33	0.37	0.36	0.36	0.40
#	12	12	12	11	12	11	12	11	11	11	10	10
MEAN	0.00	0.05	0.12	0.21	0.26	0.30	0.31	0.34	0.34	0.33	0.35	0.35
SD	0.01	0.03	0.04	0.04	0.05	0.04	0.05	0.03	0.04	0.04	0.03	0.04
CV (%)	185.77	66.23	30.81	19.92	18.64	14.09	15.16	9.65	11.34	11.12	8.68	11.44
MAX	0.02	0.08	0.15	0.27	0.34	0.36	0.40	0.38	0.38	0.38	0.39	0.40
MIN	0.00	0.00	0.04	0.10	0.17	0.22	0.24	0.25	0.25	0.25	0.32	0.27

	SCHEDULED TIME OF SAMPLE (hours after dose)								
	8.25	8.5	9	9.5	10	11	12	14	24
SUBJ#	INHIBITION OF RED BLOOD CELL ACETYLCHOLINESTERASE (fraction inhibited)								
1	0.35	0.28	0.22	0.19	0.14	0.05	0.09	0.01	-0.01
2	0.29	0.25	0.19	0.17	0.10	0.05	0.06	0.00	-0.01
3	0.33	0.30	0.23	0.20	0.21	0.15	0.05	0.06	0.00
4	0.11 ¹	0.09 ¹	0.06 ¹	0.04 ¹	0.04 ¹	0.01 ¹	0.00 ¹	0.00 ¹	0.03
5	0.26	0.23	0.19	0.09	0.05	0.04	0.02	0.00	-0.03
6	0.31	0.23	0.21	0.16	0.12	0.07	0.08	0.07	0.04
7	0.28	0.24	0.20	0.14	0.08	0.05	0.04	0.04	-0.02
8	0.29	0.22	0.14	0.13	0.08	0.04	0.04	*	0.02
9	*	*	0.00 ¹	*	0.00 ¹	0.00 ¹	0.00 ¹	0.00 ¹	0.01
10	0.34	0.28	0.20	0.17	0.14	0.10	0.06	0.05	0.03
11	0.25	0.23	0.15	0.10	0.06	0.08	0.04	0.07	-0.04
12	0.31	0.30	0.21	0.21	0.12	0.07	0.08	0.07	-0.04
#	10	10	10	10	10	10	10	9	12
MEAN	0.30	0.26	0.19	0.16	0.11	0.07	0.06	0.04	0.00
SD	0.03	0.03	0.03	0.04	0.05	0.03	0.02	0.03	0.03
CV (%)	10.80	11.90	15.25	25.73	43.20	50.80	43.74	70.59	2392.64
MAX	0.35	0.30	0.23	0.21	0.21	0.15	0.09	0.07	0.04
MIN	0.25	0.22	0.14	0.09	0.05	0.04	0.02	0.00	-0.04

* No result, due to technical problems or no sample obtained

1 Inhibition not included in the statistics. Infusion terminated early due to technical difficulties

TABLE 13

Maximal Inhibition^a of Erythrocyte Acetylcholinesterase
with Intravenous Pyridostigmine Bromide

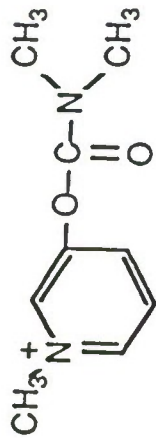
<u>Subject #</u>	<u>12.5 ug/minute*</u>	<u>18.75 ug/minute**</u>
1	0.28	0.38
2	0.26	0.36
3	0.38	0.40
4	0.28	0.34 ^b
5	0.29	0.34
6	0.27	0.36
7	0.25	0.35
8	0.30	0.34
9	0.27	0.30 ^b
10	0.31	0.38
11	0.32	0.32
12	0.32	0.40
RANGE	0.25-0.38	0.30-0.40
MEAN	0.29	0.36
SD (%)	0.03	0.03

* 6000 mcg given at steady infusion rate over 8 hours (480 minutes).

** 9000 mcg given at steady infusion rate over 8 hours (480 minutes).

^a Fractional inhibition compared to baseline erythrocyte
acetylcholinesterase activity

^b Infusion terminated early due to technical difficulties

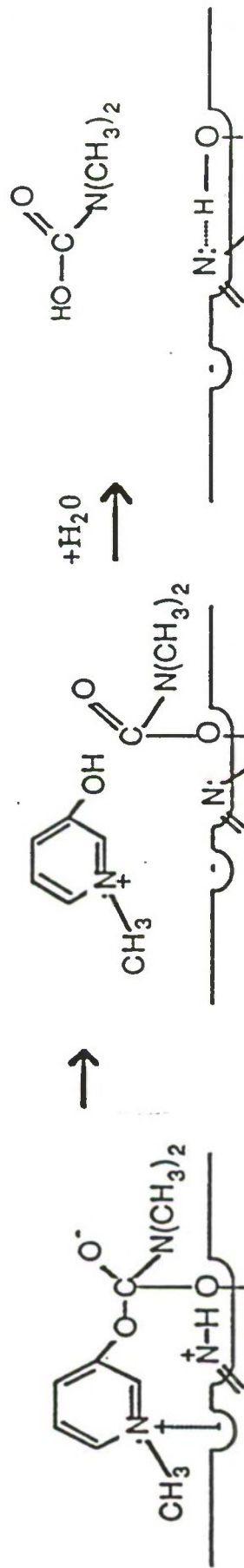


Pyridostigmine

Pyridostigmine

3-hydroxy-N-methyl pyridinium

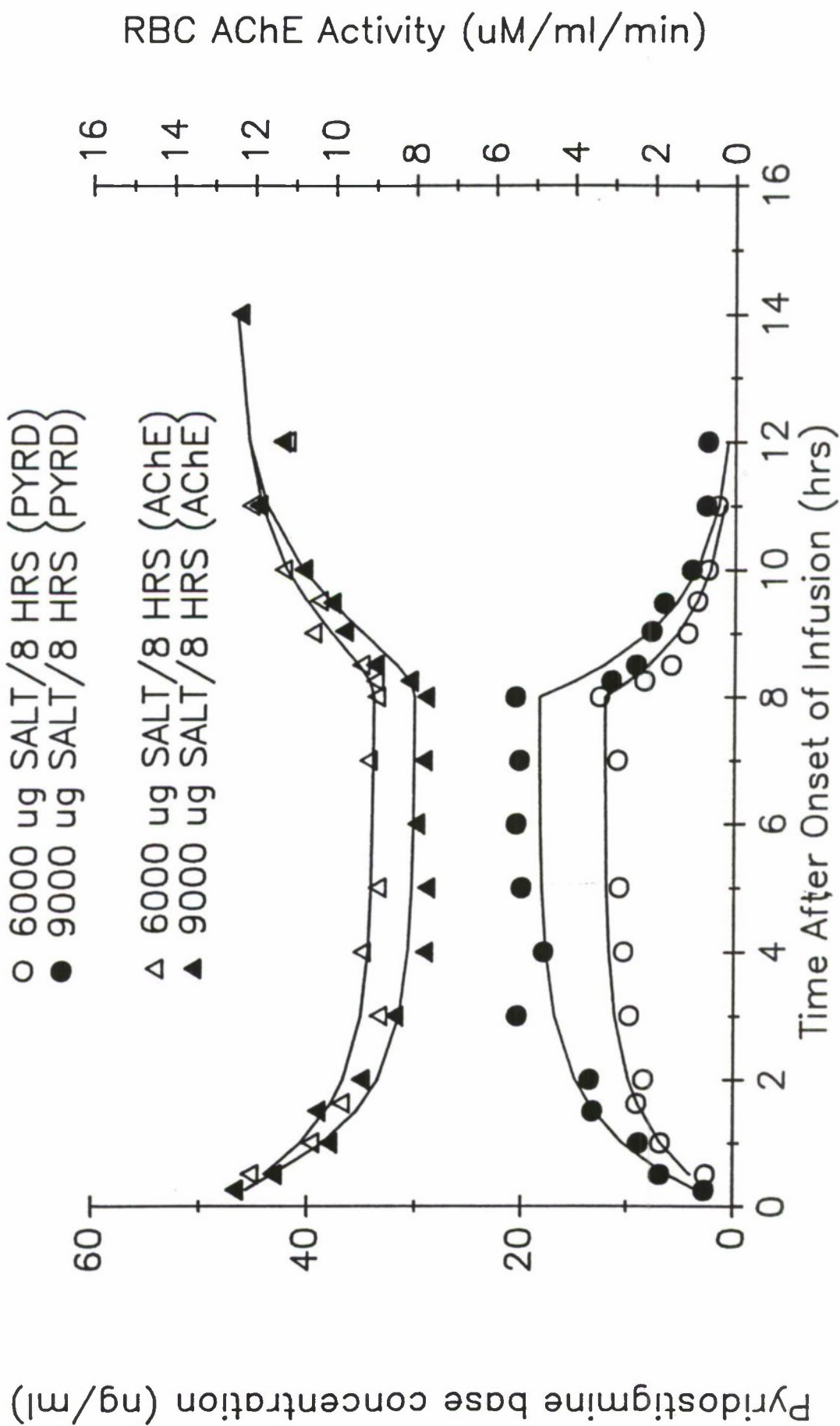
N,N-Dimethylcarbamic Acid



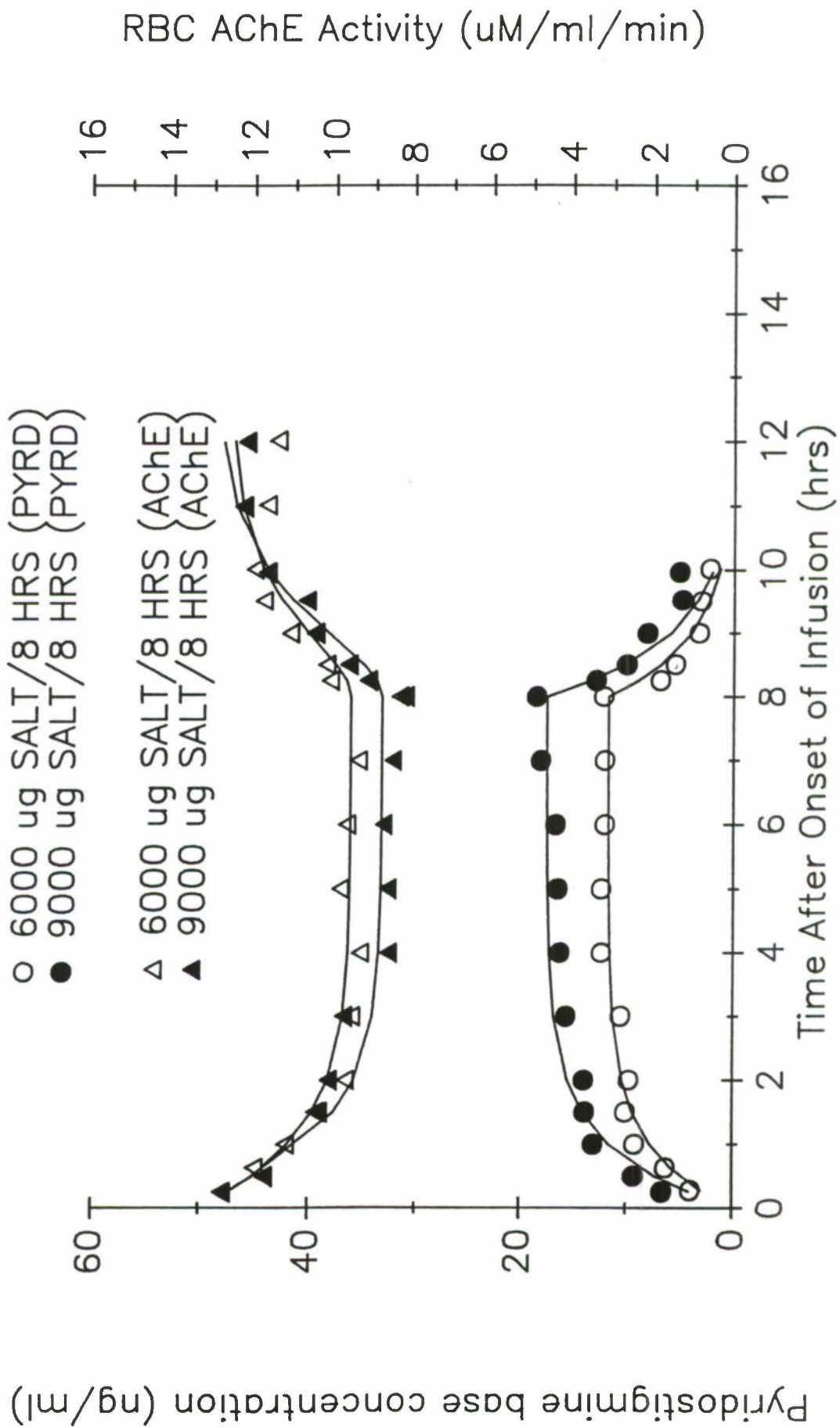
Enzyme -Inhibitor
Intermediate

Carbamylated
Enzyme

Pyridostigmine base concentrations and RBC AChE Inhibitions After 8 Hour Infusions in Subject #01

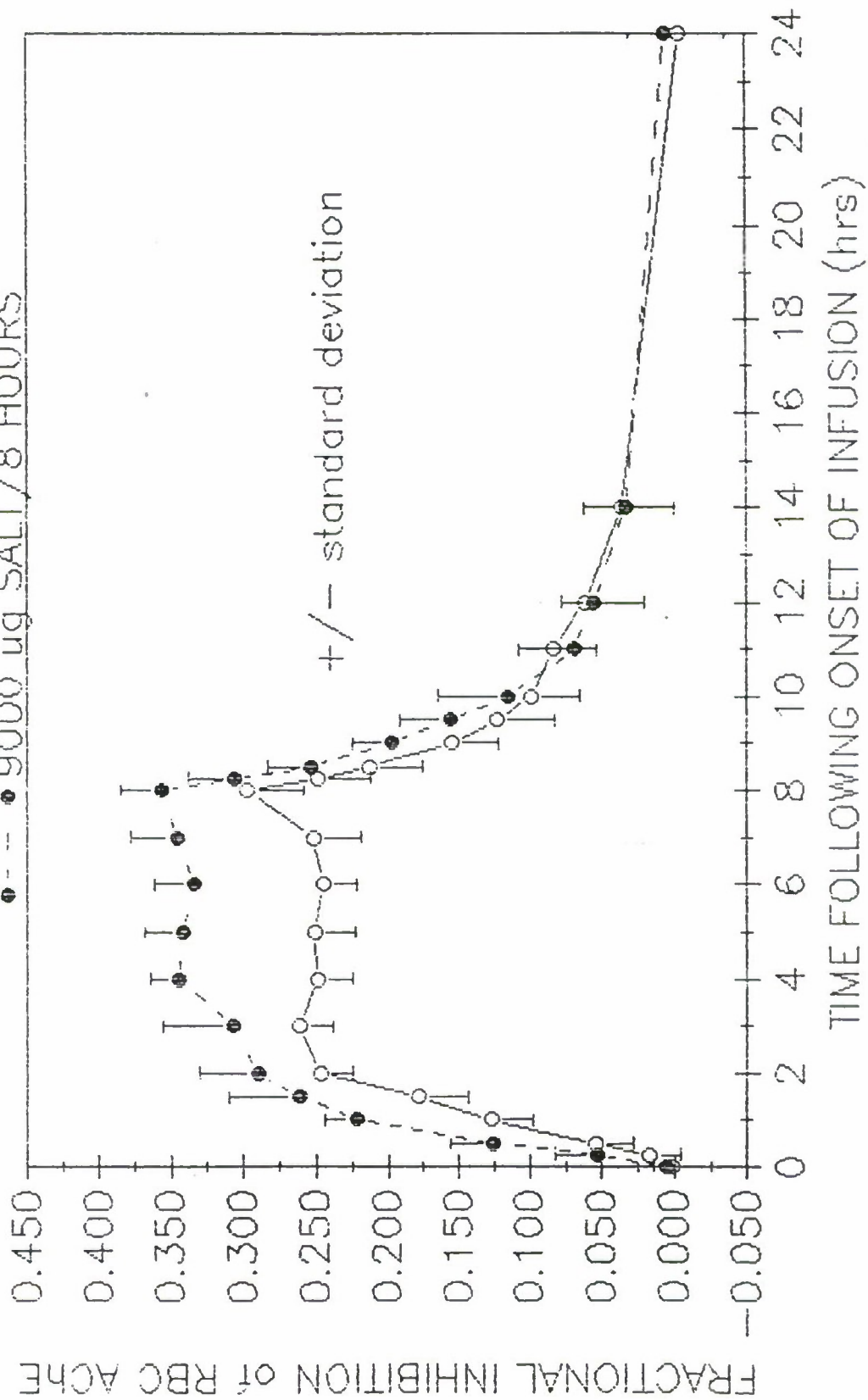


Pyridostigmine base concentrations and RBC AChE Inhibitions After 8 Hour Infusions in Subject #02



8 HOUR PYRIDOSTIGMINE INFUSION
AVERAGE OF SUBJECTS 1 THROUGH 10

○ --- ○ 6000 ug SALT/8 HOURS
● --- ● 9000 ug SALT/8 HOURS



CLINICAL INVESTIGATION CONSENT FORM
The Johns Hopkins Medical Institutions

Title of Research Project:

Pharmacokinetics and Pharmacodynamics of Sustained,
Low-dose, Intravenous Infusions of Pyridostigmine

Patient I.D. Plate

Explanation of Research Project to Subject:

You are invited to participate in a study of a drug called pyridostigmine. Pyridostigmine is a medicine that has been prescribed for patients for over 20 years. This drug is used routinely in several daily doses for years in treating patients with a disease called myasthenia gravis. This drug, based on studies in animals, may also be effective pre-treatment for accidental poisoning with certain insecticides which work in ways similar to nerve gases. This use of the drug is considered investigational by the Food and Drug Administration. The U.S. Army is funding this study to obtain information regarding the potential utility of pyridostigmine in the prevention of nerve gas toxicity. This study is designed to see how high the drug level is in the blood stream when given into your vein slowly over 8 hours and how effective the levels are in changing a blood test that may relate to the degree of protection from poisoning. The study will also determine the effect the drug may have on the muscles of the eye controlling the pupil (the round black portion in the center of the eye).

If you agree to join this study you will be hospitalized for 5 days. You will receive a dose of pyridostigmine over 8 hours through your vein by means of a mechanical pump on two separate days. Blood samples will be drawn on those days through a "heparin lock." This allows us to take repeated blood samples without sticking a new needle through your skin into a vein each time. The total blood taken for the entire study will be about a pint which is the same amount taken if you donated blood at a blood bank. You will also have pictures taken of your eyes seven times in each of the days that you receive the drug to measure the effect of the drug on the muscles which control the size of the pupil.

We believe that the risks of participation in this trial are small. The dose of the drug you will receive is much smaller than the dose usually used in treating patients with myasthenia gravis. Patients take over 20 times more of the drug each day than you will in this study. These patients sometimes develop nausea, vomiting, diarrhea, abdominal cramps, and increased body secretions. Because the dose you receive is so much smaller than what doctors use in patients, it is not likely that you will develop these symptoms. If symptoms do occur that could be related to the drug, the intravenous infusion will be turned off and the delivery of drug into your bloodstream would stop immediately. Furthermore, treatment is available if symptoms occur and become severe.

Recently, a possible new toxicity in rats was discovered which may be related to the drug. The rats were given pyridostigmine in a higher dosage than the one planned for this study. A change in muscle tissues in the area where the nerves and muscles meet was seen using an electron microscope. The significance of this finding is unknown, but it is generally accepted in the medical field that pyridostigmine is safe for man at the doses to be used in this study. Further

If you sign this form, you are willing to join the research project described to you on the other side of this page. Your doctors, or the investigators, did explain the other kinds of treatment that are available to you and to others. You should ask the principal investigator listed below any questions you may have about this research study. You may ask him/her questions in the future if you do not understand something that is being done. The investigators (or doctors) will share with you any new findings that may develop while you are participating in this study.


The records from this research study will be kept confidential and will not be given to anyone who is not helping on this study, unless you agree to have the records given out. If the study uses a new drug or device that is under the jurisdiction of the Food and Drug Administration (FDA), the FDA government officials may look at the relevant part of your medical records as part of their job to review new drug and device studies.

If you want to talk to anyone about this research study because you think you have not been treated fairly, or think you have been hurt by joining the study, or you have any other questions about the study, you should call the principal investigator, Dr. David M. Kornhauser, at (301) 955-3100, or call the Office of the Joint Committee on Clinical Investigation at 955-3008. Either the investigator or the people in the Committee office will answer your questions and/or help you to find medical care for an injury you feel you have suffered. The Johns Hopkins University, The Johns Hopkins Hospital, , and the Federal Government do not have any program to provide compensation to you if you experience injury or other bad effects which are not the fault of the investigators.

You may withdraw from the research study at any time. Even if you do not want to join the study, or if you withdraw from it, you will still have the same quality of medical care available to you at Johns Hopkins.

If you agree to join this study, please sign your name below.

NOT VALID WITHOUT THE
COMMITTEE STAMP OF
CERTIFICATION



VOID ONE YEAR FROM ABOVE DATE
RPN No. 87-06-05-07

Subject's signature
(Including children, when applicable)

Signature of Parent or Guardian (when applicable)

Witness to Consent Procedures*

Signature of Investigator

Date

*Optional unless subject is illiterate, or unable to sign.

NOTE: Signed copies of this consent form must be a) retained on file by the Principal Investigator; b) deposited in the patient's medical record; and c) given to the patient.

testing in animals is being done assess this nerve-muscle effect. Results to date indicate that the nerve-muscle effect associated with single acute doses of pyridostigmine in rats is of short-term duration and reversible. The appearance of the effect is directly related to the amount of effect on the blood test mentioned above that we will be monitoring carefully in you. Preliminary results of oral multiple dose tests now being done in dogs and rats further support these single-dose findings. If final results of these tests become available during this study, you will be informed of them. Until these tests are completed, a small risk of developing these changes cannot be ruled out for participants in this study. However, all experience with the clinical use of pyridostigmine in man over the past 20 years has not led to any reports of nerve-muscle difficulties attributed to or resulting from use of this drug.

You are under no obligation to participate in this project. Should you decide not to participate or should you decide to withdraw during the course of the project, your future care at Hopkins will not be affected. Benefits to you are primarily financial, but another potential asset is the comprehensive evaluation which accompanies this project, the results of which will be available in the future.

The records from this research study are kept confidential to the extent allowed by law. Certain regulatory groups are allowed to review them to assure that proper procedures are being followed. In addition to the FDA, authorized representatives from the Army may inspect the records related to your participation in this study.

Successful completion of the entire study will pay \$360. You will be paid by check at the time you leave the hospital.

Under Army regulations you are authorized all necessary care which is the direct result of your participation in this research and in accordance with this protocol. The medical treatment provided might include, if necessary, laboratory tests, x-rays and other procedures used in diagnosis and treatment. No other compensation is offered.

APPENDIX II

CONDITIONS FOR CHANGING PYRIDOSTIGMINE DOSE*

1. During each infusion, red blood cell acetylcholinesterase (RBC AChE) levels will be measured and assayed immediately. If the results indicate that there is >70% inhibition, that infusion will be stopped. Blood sampling times and physiologic monitoring will be adjusted so that measurements will be made with the same frequency as planned for the time period following the 8 hours infusion, with the possibility of extending those measurements to 14 hours after the beginning of the infusion.
2. If RBC AChE inhibition reaches 55% or more during the first infusion, the subject will not receive a higher dose for the second infusion. Instead, the second infusion will be 75% of the first infusion, or 4500 ug/8 hr.

*Pyridostigmine infusion rates have been calculated using data from the earlier study. Mean pyridostigmine clearance is 780 ml/min (range 380-1700 ml/min) while the IC₅₀ concentration for inhibition of enzyme activity averages about 30 ng/ml (range 17-41 ng/ml).

APPENDIX III

SCHEDULE FOR BLOOD SAMPLES FOR PYRIDOSTIGMINE LEVELS AND RBC AChE LEVELS

Time after start of infusion:

<u>Hours</u>	<u>Minutes</u>
0	0
0	15
0	30
1	0
1	30
2	0
3	0
4	0
5	0
6	0
7	0
8	0
8	15
8	30
9	0
9	30
10	0
11	0
12	0
14	0
24	0

ASSAY OF ERYTHROCYTE ACETYLCHOLINESTERASE AT THE JOHNS
HOPKINS UNIVERSITY DIVISION OF CLINICAL PHARMACOLOGY

The determination of erythrocyte acetylcholinesterase (AChE) follows the protocol established by the U.S. Army Medical Research Institute for Chemical Defense for the determination of erythrocyte AChE.

Principle of Method, Chemicals, Equipment and Materials,

Preparation of Reagents

The principles of the methods, the chemicals, materials, and equipment needed, the preparation of reagents and standards are detailed in the Standard Operating Procedure (SOP) of 18 June 1985 of the Analytical Chemistry Branch, USAMR, ICD, Aberdeen Proving Ground, Maryland 21010. These are followed exactly except for preparation of the stock glutathione solution, page 3, number 6 under A. Reagent. The typographical error is corrected and 1.844 g of glutathione, reduced form, (GSH) are dissolved in a total volume of 100 ml of EDTA diluent.

Collection and Preparation of Specimen for Sampling

Blood is collected into 3 ml purple topped Vacutainer^R (EDTA) through a catheter inserted into an arm vein. After the sample is drawn, it is mixed and brought promptly to the lab at room temperature. One and one half ml of the blood is centrifuged for 2 minutes at 15,000 RPM in an Eppendorf model 5414 centrifuge. After centrifugation all plasma is removed from the top of the packed erythrocytes with a Pasteur pipet. The erythrocytes are then picked up in a clean Pasteur pipet,

starting from the bottom of the centrifuge tube, avoiding drawing air after all the cells have been pipetted. The packed cells are transferred to a 0.5 ml sample cup and are ready for AChE analyses. Sample preparation time is kept to 3 minutes.

Preparation of Standards

Standards are prepared according to the SOP of the ICD.

Preparation of AChE Control Material

A quality control enzyme standard is used to measure assay precision. The enzyme used is electric eel AChE which is diluted in a large volume to a specific activity and frozen in aliquots. An aliquot is used each day the assay is performed. Electric eel samples were assayed in accordance with ICD SOP. See below for specific details.

Analysis Start-up

ICP SOP is followed. The heating circulation is turned on. We allow temperature to reach 37°C and verify constancy periodically.

Proportioning Pump

ICD SOP is followed. Water with Brij 35 precedes the reagents. A good bubble pattern is verified.

Colorimeter and Recorder

ICD SOP is followed. Lamp warm-up is at least 10 minutes. Once reagents are running, the recorder is zeroed for no signal and full scale. Speed is set to 1.0 cm/min.

Sampler

ICP SOP is followed. Proper operation of sampler is verified prior to running any samples.

Analyses

Recorder baseline is set to zero.

Manifold has been modified so that the substrate blank side pumps saline continuously. Erythrocytes enter only one side and one photo cell. At the end of the day, a substrate blank is run by placing the substrate line into saline and measuring the activity of the erythrocytes without a substrate. ICD SOP is followed.

Incubation time is measured. The length of time it takes erythrocytes to travel from the point where substrate is added to the sample stream until exit from the dialyzer is defined as the incubation time.

GSH standards and electric eel AChE are assayed first. A 60 micromoles/ml (u moles/ml) GSH standard is assayed followed by 15, 30, and 45 u moles/ml GSH standards, and a 1:1 dilution of the electric eel quality control. Machine sensitivity is adjusted using the STD CAL knob so that the 60 u moles/ml

standard reads 80 to 90 on the recorder scale. Two standard curves and a minimum 3 electric eel samples are assayed prior to assay of erythrocyte samples. Machine performance is standardized periodically throughout the day by running standard curves and electric eel samples. Gain is decreased if the 60 u moles/ml GSH rises above 90. Where possible, standards are assayed hourly and erythrocyte samples measured in duplicate.

Shut Down SOP

ICD is followed. Care is taken to dry the tubes and to insure that they are in a relaxed position.

Data Reduction

AChE activity is determined as follows:

The concentration of each GSH standard is divided by the peak height of the deflection produced. This value is then divided by the time of incubation in minutes to produce a factor of u moles/ml/min/chart unit. The individual factors from each standard in the curve are averaged. The average factor, u moles/ml/min/chart unit, is used to convert the deflection produced by samples to AChE activity, u moles/ml/min.

ASSAY PERFORMANCE AT JOHNS HOPKINS HOSPITAL

Quality Control

A standard solution of electric eel acetylcholinesterase is measured several times on the days that assays are performed.

The current batch of JHH electric eel was prepared on 14 October 1985 using Sigma Co., Type VI-S Cholinesterase, Acetyl (catalog #C3389) from electric eel, Lot #83F8100. One 10,000 unit vial was diluted in Tris buffer containing 1% bovine serum albumin. The dilution target was such that a one to one, dilution of the solution should contain 15.0 u moles/ml/min of cholinesterase activity.

After the dilution, aliquotting and freezing of the cholinesterase solution was performed, it was discovered that the colorimeter was not functioning correctly and that the GSH standards were in error. New standards were made; the colorimeter was repaired. The instrument was calibrated with GSH standards and the Aberdeen quality control standard. The latter's activity was 7.46 u moles/ml/min, within the range at ICD, Aberdeen.

The assay was used on 13 different days. On each day an aliquot of the electric eel quality control standard was thawed and assayed several times. The overall statistics of 127 samples from the 13 aliquots show mean activity 9.88 u moles/ml/min with a standard deviation of 0.27 u moles/ml/min. The coefficient of variation is 2.7%. Looking only at the means of the assays in a single day, the activity of the quality control is 9.91 ± 0.23 u moles/ml/min (C.V. = 2.3%).

Precision

Blood samples were drawn from 6 study subjects prior to drug administration. Each sample was assayed on several occasions. The results are tabulated in the Table.

The intra-assay coefficient of variation ranged from 0.5 to 3.5%, with a mean of 2.5%. Day to day variations in the measurement of the subject's drug free erythrocyte AChE ranged from 0.14 to 0.67 μ moles/ml/min or 1.08 to 5.48% of the value on a single day. This variation is about twice the intra-assay variation and suggests that either the inter-assay variation is greater than that of the intra-assay and/or the activity of erythrocyte AChE drawn from a single subject on different days varies to a small degree.

With a coefficient of variation of 2.5% in the assay, inhibition of enzyme activity of 5% or more probably represents real inhibition by pyridostigmine, not random variation due to the assay.

TABLE - APPENDIX G

ERYTHROCYTE ACETYLCHOLINESTERASE ACTIVITY IN 6 NORMAL VOLUNTEERS

Intra-Assay and Inter-Assay Results

Subject	Day	n	AChE u moles/ml/min (\pm S.D.)	C.V. %	b-a u moles/ml/min	(b-a)/b %
A	a	7	12.90 \pm 0.31	2.4	0.14	1.08
	b	9	13.04 \pm 0.31	2.4		
B	a	10	13.82 \pm 0.37	2.7	0.66	4.78
	b	8	14.48 \pm 0.55	3.8		
C	a	8	12.59 \pm 0.36	2.9	0.69	5.48
	b	8	13.28 \pm 0.31	2.3		
D	a	4	13.20 \pm 0.52	3.9	0.67	5.08
	b	5	13.87 \pm 0.07	0.5		
E	a	4	13.35 \pm 0.43	3.2	0.51	3.82
	b	6	13.86 \pm 0.33	2.4		
F	a	4	14.26 \pm 0.36	2.5	0.60	4.21
	b	6	14.86 \pm 0.20	1.3		

APPENDIX V
STUDY FLOW CHART

<u>DAY</u>	<u>HOURL</u>	<u>APPROXIMATE TIME</u>	<u>PROCEDURE</u>
1	-21	11:00 AM	Subjects admitted to Unit Sign Consent Form Blood: CBC with differential SMA-6 SMA-12 CPK
2	-1	7:00 AM	Insert heparin lock for blood sampling Physiologic monitoring
	0	8:00 AM	Blood: Pyridostigmine level RBC AChE* level Urine sample for pyridostigmine level <u>ADMINISTER TEST DRUG</u> Pyridostigmine 6000 ug IV over 8 hours by infusion pump
	0.25	8:15 AM	Blood:Pyridostigmine level, RBC AChE level
	0.50	8:30 AM	Blood: Pyridostigmine level RBC AChE level
	1.0	9:00 AM	Blood: Pyridostigmine level RBC AChE level
	1.5	9:30 AM	Blood: Pyridostigmine level RBC AChE level
	1.75	9:45AM	Physiologic monitoring
	2.0	10:00 AM	Blood: Pyridostigmine level RBC AChE level Breakfast

*RBC AChE=red blood cell acetylcholinesterase

(continued)

<u>DAY</u>	<u>HOUR</u>	<u>APPROXIMATE TIME</u>	<u>PROCEDURE</u>
2	3.0	11:00 AM	Blood: Pyridostigmine level RBC AChE level
	3.5	11:30 AM	Physiologic monitoring
	4.0	12:00 PM	Blood: Pyridostigmine level RBC AChE level
	5.0	1:00 PM	Blood: Pyridostigmine level RBC AChE level
	5.25	1:15 PM	Physiologic monitoring
	6.0	2:00 PM	Blood: Pyridostigmine level RBC AChE level Urine: void to begin first 2-hour urine collection
	7.0	3:00 PM	Blood: Pyridostigmine level RBC AChE level
	7.5	3:30 PM	Physiologic monitoring
	8.0	4:00 PM	End pyridostigmine infusion Blood: Pyridostigmine level RBC AChE level Urine: void to end first 2-hour collection; start second 2-hour collection.
	8.25	4:15 PM	Blood: Pyridostigmine level RBC AChE level
	8.5	4:30 PM	Blood: Pyridostigmine level RBC AChE level

(continued)

<u>DAY</u>	<u>HOUR</u>	<u>APPROXIMATE TIME</u>	<u>PROCEDURE</u>
2	9.0	5:00 PM	Blood: Pyridostigmine level RBC AChE level
	9.25	5:15 PM	Physiologic monitoring
	9.5	5:30 PM	Blood: Pyridostigmine level RBC AChE level
	10.0	6:00 PM	Blood: Pyridostigmine level RBC AChE level Urine: Void to end second 2-hour urine collection.
	11.0	7:00 PM	Blood: Pyridostigmine level RBC AChE level
	11.5	7:30 PM	Physiologic monitoring
	12.0	8:00 PM	Blood: Pyridostigmine level RBC AChE level
	14.0	10:00 PM	Blood: Pyridostigmine level RBC AChE level
3	24.0	8:00 AM	Blood: Pyridostigmine level RBC AChE level Chemistry, Hematology Physiologic monitoring
4	-1	7:00 AM	Insert heparin lock for blood sampling
	0	8:00 AM	Blood: Pyridostigmine level RBC AChE level Urine sample for pyridostigmine level
			<u>BEGIN TEST DRUG INFUSION</u> Pyridostigmine 9000 ug over 8 hours by infusion pump

(continued)

<u>DAY</u>	<u>HOUR</u>	<u>APPROXIMATE TIME</u>	<u>PROCEDURE</u>
4	0.25	8:15 AM	Blood: Pyridostigmine level RBC AChE level
	0.50	8:30 AM	Blood: Pyridostigmine level RBC AChE level
	1.0	9:00 AM	Blood: Pyridostigmine level RBC AChE level
	1.50	9:30 AM	Blood: Pyridostigmine level RCB AChE level
	1.75	9:45 AM	Physiologic monitoring
	2.00	10:00 AM	Blood: Pyridostigmine level RBC AChE level
	3.0	11:00 AM	Blood: Pyridostigmine level RBC AChE level
	3.5	11:30 AM	Physiologic monitoring
	4.0	12:00 PM	Blood: Pyridostigmine level RBC AChE level
	5.0	1:00 PM	Blood: Pyridostigmine level RBC AChE level
	5.25	1:15 PM	Physiologic monitoring
	6.0	2:00 PM	Blood: Pyridostigmine level RBC AChE level Urine: Void to begin first 2-hour urine collection.
	7.0	3:00 PM	Blood: Pyridostigmine level RBC AChE level
	7.5	3:30 PM	Physiologic monitoring

(continued)

<u>DAY</u>	<u>HOUR</u>	<u>APPROXIMATE TIME</u>	<u>PROCEDURE</u>
4	8.0	4:00 PM	End Pyridostigmine infusion Blood: Pyridostigmine level RBC AChE level Urine: void to end first 2-hour urine collection; begin second 2-hour urine collection.
	8.25	4:15 PM	Blood: Pyridostigmine level RBC AChE level
	8.5	4:30 PM	Blood: Pyridostigmine level RBC AChE level
	9.0	5:00 PM	Blood: Pyridostigmine level RBC AChE level
	9.25	5:15 PM	Physiologic monitoring
	9.5	5:30 PM	Blood: Pyridostigmine level RBC AChE level
	10.0	6:00 PM	Blood: Pyridostigmine level RBC AChE level Urine: Void to end second 2-hour urine collection.
	11.0	7:00 PM	Blood: Pyridostigmine level RBC AChE level
	11.5	7:30 PM	Physiologic monitoring
	12.0	8:00 PM	Blood: Pyridostigmine level RBC AChE level
	14.0	10:00 PM	Blood: Pyridostigmine level RBC AChE level
5	24.0	8:00 AM	Blood: Pyridostigmine level RBC AChE level Chemistry, Hematology Physiologic monitoring Discharge

CLINICAL LABORATORY NORMAL VALUES

The ranges of these values have been determined and are utilized by the Department of Laboratory Medicine of The Johns Hopkins Hospital.

<u>Serum Chemistry</u>	<u>Normal Limits</u>		<u>Units</u>
<u>Tests</u>	<u>Lower</u>	<u>Upper</u>	
Sodium	135	148	mEq/l
Potassium	3.5	5.0	mEq/l
Chloride	96	109	mEq/l
Carbon dioxide	24	30	mEq/l
Serum urea nitrogen	12	25	mg/dl
Creatinine	0.4	1.5	mg/dl
Glucose	70	115	mg/dl
Calcium	9.0	10.5	mg/dl
Total bilirubin	0.3	1.2	mg/dl
Direct bilirubin	0.1	0.4	mg/dl
Total protein	6.0	8.5	g/dl
Albumin	3.2	5.3	g/dl
Aspartate aminotransferase	0	35	IU/l
Alanine aminotransferase	0	30	IU/l
Alkaline phosphatase	30	120	IU/l
Phosphate, inorganic	3.0	4.5	mg/dl
Lactic dehydrogenase	0	220	IU/l
Creatine kinase	0	160	IU/l
Uric Acid	2.6	7.2	mg/dl
Cholesterol	124	218	mg/dl

<u>Serum Chemistry</u>	<u>Normal Limits</u>		<u>Units</u>
<u>Hematology Tests</u>	<u>Lower</u>	<u>Upper</u>	
White blood cells	4,500	13,000	#/mm ₃
Red blood cells	4.50	5.90	million/mm ³
Hemoglobin	13.2	15.6	g/dl
Hematocrit	41.0	53.0	%
Platelets	150	350	thousand/mm ³
Reticulocytes	0.5	1.5	%
White Blood Cell Differentials:			
Bands	2	7	%
Segmental Neutrophils	28	78	%
Lymphocytes	28	48	%
Monocytes	3	10	%
Eosinophils	1	4	%
Basophils	0	1	%

APPENDIX IV

PHYSIOLOGICAL MEASUREMENTS

- A. Photometric evaluations of miotic response to changes in light intensities - The subject will be seated at a table with his head secured in a headrest. After two minutes of dark adaptation, four or five flash photographs will be taken with a telemacro lens mounted on a standard 35mm SLR camera at sixty second intervals. During the course of a series of exposures the light intensity in the room will be adjusted using a calibrated rheostat. Light intensity will be measured with an optical power meter. Subsequently, the slides from the processed film will be projected on a screen and the pupil size measured. A standard disk taped below the subject's lower eyelid will be used for calibration. The following measurements will be analyzed:

- * size of pupil
- * miotic response to graded light intensities
- * maximal mydriasis (in the dark)

The pupillary response will be measured at baseline (just prior to each pyridostigmine administration) and at the following time points once the infusion has begun:

1.75 hours, 3.5 hours, 5.25 hours, 7.5 hours,
9.25 hours, 11.5 hours and 24 hours

ADVERSE REACTION REPORT

(Drugs and Biologics)

Form Approved: OMB No. 0910-0001
Expiration Date: May 31, 1986.

FDA

CONTROL NO.

ACCESSION
NO.

REACTION INFORMATION

1. PATIENT ID/INITIALS (In Confidence)

S.C.S.

2. AGE
YRS.
21

3. SEX
M

4.-6. REACTION ONSET
MO. DA. YR.
10 16 87

8.-12. CHECK ALL
APPROPRIATE
TO REACTION

7. DESCRIBE REACTION(S) (Underline single most important clinical event or reaction term)

Loss of consciousness and minor abrasions following visual change and headache for 5 minutes. No incontinence. This occurred about 2 hours after the subject had been released from The Johns Hopkins Hospital. He had been walking around the downtown Baltimore area and was sitting at a bus stop when the incident occurred.

- ☐ DIED DUE TO REACTION
☐ TREATED WITH Rx DRUG
☐ RESULTED IN, OR PROLONGED, INPATIENT HOSPITALIZATION
☐ RESULTED IN SEVERE OR PERMANENT DISABILITY
☒ NONE OF THE ABOVE

13. RELEVANT TESTS/LABORATORY DATA

Erythrocyte acetylcholinesterase level normal prior to subject's discharge from hospital.
EEG - normal.
CBC, electrolytes, chemistry panel, serum magnesium - normal.
Toxicology screen - negative.

II. SUSPECT DRUG(S) INFORMATION

14. SUSPECT DRUG(S) (Give manufacturer and lot no. for vaccines/biologics)

None - Pyridostigmine infusion given day before

20. DID REACTION ABATE
AFTER STOPPING DRUG?
☐ YES ☐ NO ☒ NA

15. DAILY DOSE
9 mg

16. ROUTE OF ADMINISTRATION
Intravenous

21. DID REACTION
REAPPEAR AFTER
REINTRODUCTION?
☐ YES ☐ NO ☒ NA

7. INDICATION(S) FOR USE
Investigational protocol

18. THERAPY DATES (From/To)
8:00 a.m. to 4:00 p.m. 10/15/87

19. THERAPY DURATION
8 hours

III. CONCOMITANT DRUGS AND HISTORY

22. CONCOMITANT DRUGS AND DATES OF ADMINISTRATION (Exclude those used to treat reaction)

None

23. OTHER RELEVANT HISTORY (e.g. diagnoses, allergies, pregnancy with LMP etc.)

None

IV. ONLY FOR REPORTS SUBMITTED BY MANUFACTURER

24. NAME AND ADDRESS OF MANUFACTURER (Include Zip Code)

V. INITIAL REPORTER (In confidence)

26.- 26a. NAME AND ADDRESS OF REPORTER (Include Zip Code)

Brent G. Petty, M.D.
600 N. Wolfe Street
Baltimore, Maryland 21205

24a. IND/NDA. NO. FOR SUSPECT
DRUG

24b. MFR CONTROL NO.

26b. TELEPHONE NO. (Include area code)
(301) 955-8181

24c. DATE RECEIVED
BY MANUFACTURER

24d. REPORT SOURCE (Check one)
☐ FOREIGN ☐ STUDY ☐ LITERATURE
☐ HEALTH PROFESSIONAL ☐ CONSUMER

26c. HAVE YOU ALSO REPORTED THIS REACTION TO THE
MANUFACTURER?
☐ YES ☒ NO

25. 15 DAY REPORT
☐ YES ☐ NO

25a. REPORT TYPE
☐ INITIAL ☐ FOLLOWUP

26d. ARE YOU A HEALTH PROFESSIONAL?
☒ YES ☐ NO

NOTE: Required of manufacturers by 21 CFR 314.80.

Sean Smith
JHH# 228-16-68
October 16, 1987

Mr. Smith left here about 10:00 a.m., went to Fayette & Wolfe Streets, waited at bus stop about 30 minutes, caught #62 bus, got off bus downtown. Walked 2-3 blocks, went into money service place on Park Avenue. They wouldn't take a personal check, so he walked another 3 blocks to a bank on Baltimore Street (closest bank), but it was closed. He then walked across street to another money service, but it also wouldn't take a personal check. Walked to #23 bus stop on Saratoga, sat there waiting about 5 minutes. While sitting at the bus stop on a stairwell he started sweating, which he attributed to ambient heat. Then after 2-3 minutes he developed blurry, green vision and pain in the left temple. Because of the visual change and sweating he reclined back onto his elbows for about 10 seconds. The next thing he knew he was being helped up from the bottom of the side of the stairwell by an old man and a woman. Vision was still blurry, possible slight head pain, especially left forehead. He sat back down on the stairway and tried to "shake it off" (shaking head). Vision slowly returned completely to normal in about 5 minutes. He moved up the steps to the porch, and then sat Indian style on the top of the porch. An ambulance was summoned, but he didn't get in, he felt well. The attendants took his blood pressure and asked questions, determining that he didn't seem sick enough to warrant ambulance transfer and emergency treatment. A girl at the scene said he was down off the stairs about two minutes before people assisted him. There was no comment from anyone about witnessing the event. His bus came within 15 minutes and took him home. He arrived there at 12:30 p.m., and called us.

He never had a similar occurrence, never blacked out or fainted, never had a seizure, never had any serious head injuries or similar visual blurring. No incontinence of bowels or bladder with the events described above, but afterward he had a tender tongue which he thinks he bit, but there was no bleeding. He denies any exposure to food, beverage, drug, toxin, other.

PE: Abrasion left forehead, superficial.
 Abrasion bridge of nose, 1 mm deep, about 6x10 mm across.
 Minor scratches left upper lip and below nose.
 No apparent motor weakness.

Erythrocyte

Acetylcholinesterase

Activity: Normal prior to subject's departure from hospital

EEG: Telephone report -- "completely normal"

Blood tests: All normal, including serum magnesium

Toxicology

screen: Negative

October 19, 1987:

Subject cashed his check. His mother said he was feeling well. Mr. Smith later called and told our research nurse that he was feeling fine and wondered if he could get into the next study.

IMPRESSION:

Loss of consciousness, etiology unclear. With normal EEG the likelihood of seizure disorder is low. Migraine equivalent is suggested by visual disturbance and headache, but has never occurred before. Since this occurred 18 hours after the pyridostigmine infusion was discontinued, I have very strong doubts that the incident was related to pyridostigmine in any way.


Brent G. Petty, M.D.

BGP/mlw



DEPARTMENT OF THE ARMY
OFFICE OF THE SURGEON GENERAL
5111 LEESBURG PIKE
FALLS CHURCH, VA 22041-3258

REPLY TO
ATTENTION OF

November 4, 1987

SGRD-HR

SUBJECT: Protocol Entitled "Pharmacokinetics and
Pharmacodynamics of Sustained, Low-Dose,
Intravenous Infusions of Pyridostigmine,"
Submitted by David M. Kornhauser, M.D.,
Johns Hopkins University (Log No. A-4695)

David M. Kornhauser, M.D.
Division of Clinical Pharmacology
The Johns Hopkins University
School of Medicine
Baltimore, Maryland 21205

Dear Dr. Kornhauser:

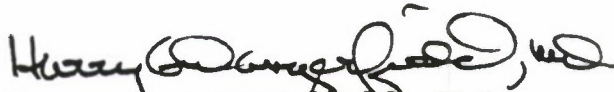
Officials at the U.S. Army Medical Research and Development Command who evaluated the incident reported under the subject protocol concur that it does not appear to be related to the administration of the test article. This incident will not be reported to the Food and Drug Administration as an Adverse Drug Reaction (ADR), but it should be referred to in the annual report for this study.

In order to satisfy our obligation to report such incidents to the appropriate Institutional Review Board (IRB), copies of this report will be made available to The Surgeon General's Human Subjects Research Review Board at its November 18, 1987 meeting. We remind you that, if you have not already done so, you should report this to your IRB.

If any additional subjects are enrolled in this study, they should be warned to make a slow return to normal activities following their release.

If you have any questions, please contact the
Human Use Review Office at (301) 663-2165.

Sincerely,



Harry G. Dangerfield, M.D.
Colonel, Medical Corps
Acting Chairman, Human Subjects
Research Review Board

Copies Furnished:

U.S. Army Medical Materiel Development Activity,

ATTN: SGRD-UMP/SGRD-UMS-B

U.S. Army Medical Research Acquisition Activity,

ATTN: SGRD-RMA-RC (85C5133)

U.S. Army Medical Research and Development Command,

ATTN: SGRD-PLA/SGRD-PLE

Walter Reed Army Institute of Research,

ATTN: SGRD-UWM (COL Schuster)

Adverse Events

Definitions of Relationship to Test Drug

Definitely Related (must have first three, and either #4 or #5)

This category applies to those adverse experiences which are considered, with a high degree of certainty, to be related to the test drug. An adverse experience may be considered definitely related to the drug if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse experience does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; e.g., 1) bone marrow depression; 2) fixed drug eruptions; and 3) tardive dyskinesias.
4. It follows a known pattern of response to the suspected drug.
5. It recurs upon rechallenge with the suspected drug.

Probably Related (must have first three)

This category applies to those adverse experiences in which are considered, with a moderate degree of certainty, to be related to the test drug. An adverse experience may be considered probably related to the drug if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse experience does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; e.g., 1) bone marrow depression; 2) fixed drug eruptions; and 3) tardive dyskinesias.
4. It follows a known pattern of response to the suspected drug.

Adverse Events (cont'd)

Possibly Related (must have first two)

This category applies to those adverse experiences in which the connection with the test drug administration appears unlikely, but cannot be ruled out with certainty. An adverse experience may be considered possible related to the drug if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the volunteer.
3. It follows a known pattern to the suspected drug.

Definitely Not

This category is applicable to those adverse experiences which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for drug relationship listed under Definitely, Probably or Possibly Related. In such cases there is no conceivable way in which the test drug could be implicated; the event is associated with a known underlying condition or is physiologically impossible as a drug side effect; or is so remote from drug exposure as to be impossible.

Unknown

Inadequate data are available to make a determination.

APPENDIX J

Personnel Receiving Contract Support

Paul S. Lietman, M.D., Ph.D.

Brent G. Petty, M.D.

David M. Kornhauser, M.D.

Steven Kuwahara, Ph.D.

Lynda Nerhood, R.N.

Amina Woods, M.S.

Nadia Badiee, B.S.

Kathy Bell, B.A.

Evelyn Fleckenstein

Mary L. Williams

Bondella Jones

Darlene Hamilton

APPENDIX K

Distribution List

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Center for Astrophysical Sciences

ULTRAVIOLET BACKGROUND RADIATION

Richard C. Henry

March 1991

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